Болест на Уолман е рядко генетично заболяване, засягащо липидната обмяна. Генетичният дефект е разположен на дългото рамо на 10 хромозома - 10q23.31. Болестта се унаследява по автозомно рецесивен път. В малък процент се срещат и de novo възникнали мутации. Заболяването спада към група, свързана с дефекти в липидното съхранение и по-конкретно със специфично ензимно нарушение на лизозомната функция. Причина за болестта е мутация на ген, отговорен за синтезата на ензима LAL /лизизомна киселa липаза/, който има ключова роля в липидния метаболизъм. Генът носи неговото име и е наречен лизозомна киселa липаза А ген /lysosomal acid lipase A gene, LIPA gene/. Липсата на ензима блокира хидролизирането на холестериловите естери и триглицеридите, което довежда до последващо отлагане на депозити от муколипиди и някои комплексни въглехидрати(мукозахариди) в редица органи като: черен дроб, слезка, надбъбречни жлези, черва, лимфни възли, костен мозък, бели дробове и др. Засягането на тези органи пряко корелира с механизмите на рецепторно-медиирана ендоцитоза и лизозомно разграждане на липопротеините. Метаболитните промени довеждат до състояние на тип 2b хиперлипидемия, характеризиращо се с повишени серумни нива на LDL-холестерол и намален HDL-холестерол.

Описват се две фенотипни форми на болестта – ранна форма на Уолман и късна (адултна) форма, още наречена болест на натрупването на холестерилови естери (Cholesteryl Ester Storage Disease, CESD).

При ранна форма на болест на Wolman има пълна липса на LAL (под 1%), което довежда до фулминантно начало и изява на заболяването още в първите седмици на новороденото или кърмаческия му период. Липсата на ензима довежда до малнутриция, с последваща малабсорбция, натрупване на холестерилови естери в макрофагите на хепатоцитите и отлагане на калцификати в надбъбречни жлези с последваща кортикална инсуфициенция. При засегнатите деца се наблюдава лош тегловен прираст, понижен мускулен тонус, повръщане, диария, забавено развитие, жълтеница. Черният дроб значително се уголемява, като бързо се развиват и чернодробните усложнения -фиброза, склероза до цироза. Лабораторно се наблюдават завишени стойности на трансаминазите, общия холестерол и в частност на LDL-холестерола. Децата често загиват в рамките на една годишна възраст от полиорганна недостатъчност. Хистологично в черия дроб се открива натрупване на огромно количество липиди в хепатоцитите, фиброза, стеатоза и микросъдови промени.

При CESD има дефицит на LAL ензима, чиято активност е частично запазена и варира в границите от 2 до 11%. Симптомите започват да се изявяват след 1 годишна възраст. Заболяването протича плавно и преживяемостта на пациентите зависи главно от темпото на отлагане на холестерина и последващите чернодробни и сърдечно съдови усложнения. Както при ранната, така и при късната форма на болестта на Wolman, се наблюдават нарушения в растежа, диария, фебрилитет, повръщане, хепато и спленомегалия, повишени серумни трансаминази, триглицериди и LDL-холестерол, драстично се понижава HDL-холестерола. Времето и тежестта на изява на симптомите са пряко свързани със запазената активност на ензима LAL при всеки конкретен пациент.

Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)

E75.5

Код на заболяването по Orpha code

Orpha75233
OMIM 278000

Епидемиологични данни за заболяването в Република България

Орфанет цитира световна честота на болестта на Уолман от 1 до 9 на 1 000 000 население.

Няма епидемиологични данни за заболяването в Република България. В нашия център до момента е диагностициран и се лекува един пациент. Известен ни е един починал пациент под 1 год. възраст (София) и един пациент в процес на диагностично доказване (Пловдив).

В т.ч. научни публикации от последните пет години и приложена библиографска справка

Няма публикации относно епидемиологичните данни на заболяването в Република България.
Прилагаме публикация, касаеща пациента, за когото се грижим ние.

1. Цочев К, Йотова В. Първият пациент адултна форма на болест на Wolman в България. Известия на СУВ, 2016

Епидемиологични данни за заболяването в Европейския съюз

Няма епидемиологични данни за заболяването в Европейския съюз.

В Орфанет се споменава честота за цеия свят от 1 до 9 пациенти на 1 млн. население.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

Няма публикации относно епидемиологичните данни за заболяването в Европейския съюз.

Интерес представлява изследването на ***Scott et al. (2013, прил.),*** които изследват здрави представители на различни раси за честотата на мутиралия алел на LIPA гена (10q23.31) - c.894G>A, който е доказано най-честата мутация при пациенти с адултна форма на болестта на Уолман – болест на натрупването на холестерилови естери (около 60% от диагностицираните случаи). Използвайки този подход, авторите достигат до предполагаема честота от около 1 на 130 000 в кавказката раса. При проучвания на хора от ирано-еврейски произход, живеещи в Лос Анджелис, е открита и публикувана най-висока честота на патологични алели – 1 на 4 200 новородени ***(Valles-Ayoub et al., 2011, Genet Test Mol Biomarkers)***. В публикация от 1999 г. чешки колектив съобщава за 13 случая на заболяването наблюдавани за 25 години ***(Elleder, 1999, Cas Lek Cesk, прил.).*** Всички тези данни показват, че се касае за изключително рядко заболяване.

Оценка на съответствието на заболяването с дефиницията за рядко заболяване съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето

Заболяването съответства на дефиницията за рядко заболяване съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето. Заболяването е хронично и инвалидизиращо по отношение на фертилността и психичното здраве и засяга по-малко от 5 на 10 000 души в Европейския съюз.

Критерии за диагностициране на заболяването

Широка гама от наследствени, сърдечно-съдови и метаболитни заболявания вземат участие в диференциално диагностичния план на заболяването. Част от тях са: полигенна хиперхолестеролемия, фамилна хиперхолестеролемия, гликогенози, автоимунен хепатит, вирусен хепатит, неалкохолна чернодробна стеатоза. Željko Reiner et al. представят алгоритъм, публикуван в Atherosclerosis 235/2014, отразяващ диагностичния план при поставяне на диагнозата болест на Wolman. Тя трябва да бъде подозирана при липса на доказателства за фамилна обремененост и наличието на данни за хепатомегалия, хиперхолестеролемия за сметка на **LDL-холестерол>4.7mmol/l** (за деца под 8 г. **LDL>3.4mmol/l**) и понижен **HDL-холестерол<1.3mmol/l**, повишен **ALT>1.5x** от референтните стойности, **BMI<30кг/м^2**, ехографска находка за фиброза или стеатоза на черния дроб. Наблюдава се нарушение в растежа, умора, болка в дясно подребрие, гадене, диария. С напредване на заболяването се наблюдават усложнения като: кахексия, жълтеница, епистаксис, подуване на корема, тромбоцитопения, анемия. В диагностичния план влизат и резултатите от активността на LAL ензима, данни от чернодробна биопсия за отлагане на мастни включвания в хепатоцитите, хипертрофирали Купферови клетки, фиброза. Наличието на калцификати в надбъбреците (потвърдено чрез КТ-изследване), както и изобилието от пенести клетки на препарат от костно мозъчна пункция е по-характерно за ранната форма на болестта на Wolman. Генетичният анализ остава златен стандарт в окончателната диагноза.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Reiner Z. et al./ Atheroclerosis 235 /21-30.2014 Apr 15 /Www.Atherosclerosis-Journal.Com/Article/S0021-9150(14)00202-0/Pdf/

2.Tylki-Szymańska1 A., A. Jurecka. Lysosomal Acid Lipase Deficiency: Wolman Disease And

Cholesteryl Ester Storage Disease/ Issn 0350-1914 Udc: 616-056.7 / Sec. Med. Sci., xxxv 1, 2014

3.Abel F., Burton BK, Billmeyer B. The Role Of Sebelipase Alfa In Treating Lysosomal Acid Lipase Deficiency (Lal-D)

4. Hoeg J.M, Demosky S.J. et al. Cholesteryl Ester Storage Disease And Wolman Disease: Phenotypic Variants Of Lysosomal Acid Cholesteryl Ester Hydrolase Deficiency. Am. J. Hum. Genet. 36: 1190-1203, 1984. [Pubmed: 6097111, Related Citations]

5. Hoffman E, Barr ML, Giovanni MA, MF Murray. Lysosomal Acid Lipase Deficiency / September 1, 2016/ Genereviews

Алгоритми за диагностициране на заболяването

Подробна фамилна анамнеза. Възможно е провеждането на тестове за носителство, както и такива за пренатална диагностика на бременни жени с повишен риск, чрез молекулярно-генетичен анализ. Анамнестични данни за нарушение в растежа, умора, болка в дясно подребрие, гадене, диария. С напредване на заболяването се наблюдават усложнения като: жълтеница, епистаксис, подуване на корема, стеаторея, тромбоцитопения, анемия. Физикален преглед: оглед и палпация на корема, определяне нивото на хепато/спленомегалия, оглед на кожата за пигментации. Пълна кръвна картина. Биохимичен анализ с особенна насоченост към ALT, AST, Общ холестерол, LDL – холестерол, HDL – холестерол, VLDL – холестерол, триглицериди. ОГТТ и Глюкагонов тест. Хормонални изследвания: TSH, IGF-1, Insulin. Образни изследвания: Костна плътност, УЗ - ехографска находка за фиброза или стеатоза на черния дроб, КТ с контрас - търсим надбъречна патология. Изследване на активността на LAL ензима е ключово при поставане на диагнозата, както и данните от евентуално проведена чернодробна биопсия за отлагане на мастни включвания в хепатоцитите, хипертрофирали Купферови клетки, фиброза. При невъзможност да се изпълни някоя от стъпките по-горе, може да се премине директно към генетичен анализ. Същият е крайният и най-достоверен начин за потвърждаване на диагнозата /Tylki-Szymańska&A Jurecka, 2014/

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1.Donna L et al. /Cholesterol ester storage disease: Review of the findings in 135 reported patients with an inderdiagnosed disease/ Journal of hepatology 2016

2.Hoffman EP, ML Barr, MA Giovanni and MF Murray. Lysosomal Acid Lipase Deficiency / September 1, 2016/ GeneReviews

3. Tylki-Szymańska1 A., A. Jurecka. Lysosomal Acid Lipase Deficiency: Wolman Disease And Cholesteryl Ester Storage Disease/ Issn 0350-1914 Udc: 616-056.7 / Sec. Med. Sci., xxxv 1, 2014

Алгоритми за лечение на заболяването

Лечението на заболяването се провежда от мултидисциплинарен екип. Първите проучвания на пациенти с CESD, провеждали терапия със статини, показват незадоволителен ефект върху нивата на HDL-холестерола и трансаминазите, въпреки постигането на добър контрол върху нивата на LDL-холестерола и триглицеридите. Чернодробната трансплантация като радикален метод на лечение е описана в единични случаи. Възникналите пост трансплантационни усложнения при по-голяма част от тези пациенти, не оправдава ползата от провеждането на такава.

След 20 годишен тестов период през декември 2015 г., се появи първият ефективен медикамент в лечението на болестта на Wolman. Препаратът, наречен Sebelipase alfa (Kanuma) е рекомбинантен ензимо-заместващ продукт, който е преминал клинични проучвания при хора и е удобрен за употреба в Европа и Америка. Sebelipase alfa катализира лизозомната хидролиза на холестериловите естери, триглицеридите, свободния холестерол, глицерол и свободните мастни киселини. Приемът на ензимо-заместващия продукт подобрява чувствителността на рецепторите за LDL, с което намалява и съдържанието на мазнини в черния дроб и нормализира трансаминазите. При деца с болест на Wolman (под 6 мес.) в зависимост от тежестта на състоянието и активността на LAL-ензима, дозата варира от 0.35mg - 3mg/kg за седмица. При деца и юноши с CESD дозата започва от 0.35mg - 1mg/kg за седмица, приложена чрез венозна инфузия с продължителност 1-2 часа. Стартирането на терапията изисква изследване на нивото на активността на LAL-ензима в организма. На базата на процента запазена активност се определя и началната терапевтична доза. Необходимо е редовното му проследяване и по

време на терапията. За момента няма алтернатива за лечението на пациенти с болестта на Wolman.

Многоцентрово двойно-сляпо плацебо-контролирано проучване ARISE (Phase 3 Acid Lipase Replacement Investigating Safety and Efficacy /ClinicalTrials.gov number, NCT01757184), завършено през януари 2016 при 66 пациента с дефицит на LAL и провели терапия със Sebelipase alfa/Kanuma, отчита значително нормализиране на стойностите на ALT/AST, понижение на LDL-холестерола и подобрение на HDL-холестерола при таргетираната, спрямо плацебо контролирана група по време на 20 седмичния период на лечение с препарата.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Tylki-Szymańska1 A., A. Jurecka. Lysosomal Acid Lipase Deficiency: Wolman Disease And Cholesteryl Ester Storage Disease/ Issn 0350-1914 Udc: 616-056.7 / Sec. Med. Sci., xxxv 1, 2014

2.Donna L et al. Cholesterol Ester Storage Disease: Review Of The Findings In 135 Reported Patients With An Inderdiagnosed Disease/ Journal Of Hepatology 2016

3.Sebelipase Alfa/Kanuma - Summary Of Product Characteristics, Http://Www.Ema.Europa.Eu. / Eu/1/15/1033/001

4.Burton B et al. A Phase 3 Trial Of Sebelipase Alfa In Lysosomal Acid Lipase Deficiency. N Engl J Med. 2015; 373(11): 1010-20

Алгоритми за проследяване на заболяването

Проследяването на заболяването, също както и лечението, се осъществява от мултидисциплинарен екип, съставен от ендокринолози, гастроентеролози, кардиолози, хематолози, специалисти по образна диагностика, лабораторни специалисти, генетици. Следат се:

- Клинично състояние, растеж и пубертетно развитие (за педиатричните пациенти), хепатоспленомегалия, вкл. с образни методи
- Нива на липиди
- Чернодробни показатели (цитолиза, синтетична и дезинтоксикационна функции, белези на цироза)
- Кръвни показатели
- Костна плътност

Докато за фамилна и от друг характер хиперхолестеролемия и хипертриглицеридемия съществуват множество гайдлайни и клинични алгоритми, за Уолман няма изградени специални, но се работи последователно, спазвайки правилата на добрата клинична практика.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Hoffman EP. Lysosomal Acid Lipase Deficiency. In: Pagon A, Adam MP, Ardinger HH, et al., Edtors. GeneReviews. Seattle (WA): University of Washington, Seattle; 1993-2017. NCBI Bookshelf. A service of the National Library of Medicine, National Institute of health
2. Цочев К, Йотова В. Първият пациент адултна форма на болест на Wolman в България. Известия на СУВ, 2016

Алгоритми за рехабилитация на заболяването

Описаните в литературата пациенти са много разнообразни, вкл. до късна и случайна диагноза в хода на уточняване произхода на значима смесена хиперлипидемия, или даже чернодробна цироза (Sojke et al., The Netherlands J Med, 2015, прил.)

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Sjouke B, van der Stappen JW, Groener JE, Pepping A, Wevers RA, Gouw A, Dikkeschei LD, Mijnhout S, Hovingh GK, Alleman MA. Hypercholesterolaemia and hepatosplenomegaly: two manifestations of cholesteryl ester storage disease. Neth J Med 2015; 73(3):129-32

Необходими дейности за профилактика на заболяването (ако такива са приложими)
Няма профилактика на заболяването. Възможна е само вторична профилактика на чернодробната цироза и предотвратяване на костномозъчна трансплантация, за момента единствено чрез лечение с Канума.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
-
Предложения за организация на медицинското обслужване на пациентите и за финансиране на съответните дейности, съобразени с действащата в страната нормативна уредба
По съществуващата в момента нормативна уредба има достатъчно възможности за диагностициране и лечение на тези пациенти. То се осъществява предимно болнично (клинични пътеки 82 и 83, а в началото за диагнозтика – КП 12). Проблеми, чието решение е наложително: • Допълнително финансиране (ниво на LAL, генетични изследвания). В момента те се изследват в чужбина и се заплащат от самите пациенти; • Организация на лечението на пациенти. На този етап оформяне на Център не е оправдано, тъй като за момента в България има само един пациент. С натрупването на опит в бъдеще ще има и такава необходимост.
Описание на опита с конкретни пациенти със съответното рядко заболяване (ако има такъв)
Опитът с подобни пациенти в Република България е малък до липсващ. Нашият мултидисциплинарен екип понастоящем проследява един пациент (приложени епикриза и публикация), който според нашата информация е единственият диагностициран пациент с адултна форма на болестта. При пациента е започнато диетично лечение, от което не се очаква ефект. Направени са съответни стъпки за осигуряване на Канума (одобрение на Центъра, compassionate use, както и решение на Фонда за лечение на деца). Очаква се решението на компания Alexion за предоставяне на препарата.

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на публикации към рядко заболяване Болест на Wolman

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Болестта на Wolman е рядко генетично заболяване, засягащо липидната обмяна. Генетичният дефект е разположен на дългото рамо на 10 хромозома – 10q23.31 (OMIM #278000)[1]. Заболяването спада към група, свързана с дефекти в липидното съхранение и по-конкретно със специфично ензимно нарушение на лизозомната функция. Причина за болестта е мутация на ген, отговорен за синтезата на ензима LAL /лизизомна киселa липаза/, който има ключова роля в липидния метаболизъм.[2] Генът носи неговото име и е наречен лизозомна киселa липаза А ген /lysosomal acid lipase A gene, LIPA gene/. Липсата на ензима блокира хидролизирането на холестериновите естери и триглицеридите, което довежда до последващо отлагане на депозити от муколипиди и някои комплексни въглехидрати (мукозахариди) в редица органи като: черен дроб, слезка, надбъбречни жлези, черва, лимфни възли, костен мозък, бели дробове и др. Засягането на тези органи пряко корелира с механизмите на рецепторно-медиирана ендоцитоза и лизозомно разграждане на липопротеините.[3,4,5] При здрави хора в черния дроб има специфични рецептори за LDL. Те улавят и свързват LDL-холестерола, разпознавайки го чрез специфичен за тях белтък апоВ-100. Чрез ендоцитоза цялостният комплекс (LDL-холестерол+рецептор) навлиза в хепатоцита, където се слива с лизозомите, съдържащи различни хидролази. При нормални условия, LAL би хидролизирал липидите и освободеният холестерол би могъл да се използва за различни функции на клетката, основно като субстрат в различни видове синтези.[6] Липсата на свободен холестерол в хепатоцита блокира лизозомното захващане на холестеринови естери, което довежда до потискане на механизма на обратно захващане на HMG-COA (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase) редуктазата, с което се повишава синтезата на холестерол. Също така се стимулира синтезата на АроВ и VLDL чрез повишаване на LDL-рецепторната чувствителност на повърхността на клетъчната мембрана. Всички тези метаболитни промени довеждат до състояние на тип 2b хиперлипидемия, характеризиращо се с повишени серумни нива на LDL-холестерол и намаление на HDL-холестерола.[7,8,9]

През 1956 г. Wolman за първи път описва ранната форма на болестта при кърмаче от близкородствен брак, като го свързва със симптоми на повръщане, диария, хепато-спленомегалия и калцификати в надбъбречни жлези.[10] Малко по-късно през 1963 г. дефицитът на LAL е открит във фибробласти от Fredrickson, който диагностицира и първия пациент с адултна (късна) форма на болест на Wolman. Тя получава названието ***болест на натрупването на холестеринови естери*** /Cholesteryl Ester Storage Disease - CESD/.[11] Редица публикации подробно описват фенотипната вариабилност и клиничната хетерогенност, характеризираща се от различните алелни мутации на гена, отговорен за синтеза на LAL и по-специално пациентите със CESD.[12,13,14]

В световен мащаб има данни за не повече от 135 случая на CESD[9], като за България няма данни да е описвана някоя от двете форми на болестта на Wolman. Честотата на заболяването пряко корелира с етническата принадлежност и географския регион, като варира в различните популации в границите от 1 на 40 000 до 1 на 300 000 новородени.[15] Изследване сред общността в Лос Анжелис открива по-голяма предиспозиция на семействата от еврейски произход с ирански корени – 1 на 4200[16]. В обзор, направен от Donna L et al.[9], обхващащ 135 пациента с късна форма на болестта се посочва по-голям превес на заболели сред Европейската (65 пациента), Северно Американската (17 пациента) и Южно Американската популация (14 пациента). Възрастта от 3 до 5 години се представя като пикова за поставяне на диагнозата. При

89% от обхванатите, тя е била направена на възраст под 12 години. При 99,3% от участниците в цитираното проучване е била налична хепатомегалия, а при 74% и спленомегалия. Авторите наблюдават по-ранна изява и бавна прогресия на усложненията при пациентите с мутации в екзон 8 на LIPA гена.

Заболяването се унаследява по автозомно рецесивен път, като всеки един от родителите предава мутантно копие от гена. В малък процент се срещат и de novo възникнали мутации. Възможно е провеждането на тестове за носителство, както и такива за пренатална диагностика на бременни жени с повишен риск, чрез молекулярно-генетичен анализ[16].

При ранна форма на болест на Wolman има пълна липса на LAL (под 1%)[9], което довежда до фулминантно начало и изява на заболяването още в първите седмици на новороденото или кърмаческия му период. След плавен етап на адаптация и регулярно хранене, настъпва срив в общото състояние на децата. Липсата на ензима довежда до малнутриция, с последваща малабсорбция, натрупване на холестеринови естери в макрофагите на хепатоцитите и отлагане на калцификати в надбъбречни жлези с последваща кортикална инсуфициенция.[17,18] При засегнатите деца се наблюдава лош тегловен прираст, понижен мускулен тонус, повръщане, диария, забавено развитие, жълтеница, увеличен черен дроб с последващи фиброза, склероза до цироза. Слезката прогресивно се уголемява, като в рамките на 1-2 месеца може да нарастне до 20 пъти.[4] Лабораторно се наблюдават завишени стойности на трансаминазните показатели, общия холестерол и в частност на LDL-холестерола. Децата често загиват в рамките на една годишна възраст от полиорганна недостатъчност. Хистологично в черия дроб се открива натрупване на огромно количество липиди в хепатоцитите, фиброза/стеатоза и микросъдови промени. Отличителна черта е наличието на хипертрофирали Купферови клетки и портални макрофаги с пенлив вид. Типична находка е изобилието от пенести клетки в костен мозък и вакуолизирани лимфоцити в периферната циркулация .[19,12]

При CESD има дефицит на LAL ензима, чиято активност е частично запазена и варира в границите от 2 до 11%.[20] Симптомите започват да се изявяват след 1 годишна възраст. Заболяването протича плавно и преживяемостта на пациентите зависи главно от темпото на отлагане на холестерина и последващите чернодробни (фиброза, склероза, цироза) и сърдечно съдови усложнения. Най-честата причина за смърт при тази късна форма на болестта е чернодробната недостатъчност. Обстойни проучвания на дефекти в гена за LAL и по-конкретно тези в екзон 8, посочват предпоставки за поява на ранни артеросклеротични промени в съдовете и по-голям риск от развитието на миокарден инфаркт при носителите на този вид мутация.[21] Както при ранната, така и при късната форма на болестта на Wolman, се наблюдават нарушения в растежа, диария, фебрилитет, повръщане, хепато и спленомегалия, повишени серумни трансаминази и LDL-холестерол. Времето и тежестта на изява на симптомите са пряко свързани със запазената активност на ензима LAL при всеки конкретен пациент. Калцификати в надбъбреците се срещат в късния период на развитие на заболяването и то само при 2/3[2] от диагностицираните.

Широка гама от наследствени, сърдечно-съдови и метаболитни заболявания вземат участие в диференциално диагностичния план на заболяването. Част от тях са: полигенна хиперхолестеролемия, фамилна хиперхолестеролемия, гликогенози, автоимунен хепатит, вирусен хепатит, неалкохолна чернодробна стеатоза. Željko Reiner et al.[22] представят алгоритъм, изработен от Европейски екип и публикуван в Journal of Hepatology 2016, отразяващ диагностичния план при поставяне на диагнозата болест на

Wolman. Тя трябва да бъде подозирана при липса на доказателства за фамилна обремененост и наличието на данни за хепатомегалия, хиперхолестеролемия за сметка на **LDL-холестерол>4.7mmol/l** (за деца под 8 г. **LDL>3.4mmol/l**[23]) и понижен **HDL-холестерол<1.3mmol/l**, повишен **ALT>1.5x** от референтните стойности, **BMI<30кг/м2**, ехографска находка за фиброза или стеатоза на черния дроб. От клиничната картина първостепенно значение имат данните за нарушение в растежа, умора, болка в дясно подребрие, гадене, диария. С напредване на заболяването се наблюдават усложнения като: жълтеница, епистаксис, подуване на корема, тромбоцитопения, анемия. В диагностичния план влизат и резултатите от активността на LAL ензима, данни от чернодробна биопсия за отлагане на мастни включвания в хепатоцитите, хипертрофирали Купферови клетки, фиброза. Наличието на калцификати в надбъбреците (потвърдено чрез КТ-изследване), както и изобилието от пенести клетки на препарат от костно мозъчна пункция е по-характерно за ранната форма на болестта на Wolman. Генетичният анализ остава златен стандарт в окончателната диагноза.[22,4]

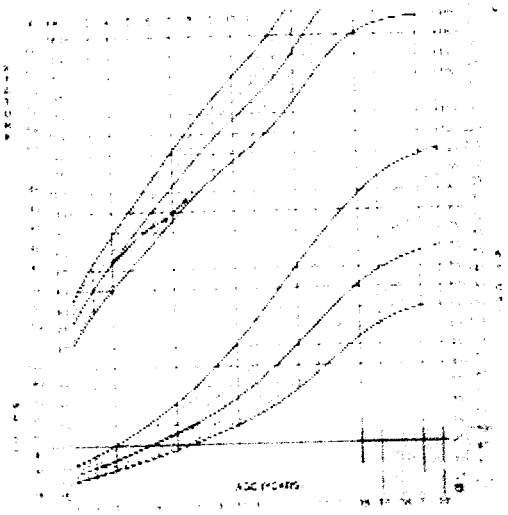
Първите проучвания на пациенти с CESD, провеждали терапия със статини, показват незадоволителен ефект върху нивата на HDL-холестерола и трансаминазите, въпреки постигането на добър контрол върху нивата на LDL-холестерола и триглицеридите.[24] Обзор в Journal of Hepatology от 2016 подчертава, че "статините нямат ефект в подобряването на чернодробните показатели или в предотвратяването на чернодробните увреждания".[9] Двугодишно проследяване на пациенти с LAL-дефицит, провели терапия с ловастатин, също достига до това заключение и отхвърля ползата от лечението.[7] Чернодробната трансплантация като радикален метод на лечение е описана в единични случаи. Възникналите посттрансплантационни усложнения при по-голяма част от тези пациенти, не оправдава ползата от провеждането на такава.[12,25,]

След 20 годишен тестов период през декември 2015 г., се появи първият ефективен медикамент в лечението на болестта на Wolman. Препаратът, наречен Sebelipase alfa на Kanuma[26] е рекомбинантен ензимо-заместващ продукт, който е преминал клинични проучвания при хора и е удобрен за употреба в Европа и Америка. Медикаментът се произвежда от яйчен белтък на трансгенни пилета Gallus, чрез рекомбинантна ДНК (рДНК) технология. Sebelipase alfa катализира лизозомната хидролиза на холестериновите естери, триглицеридите, свободния холестерол, глицерол и свободните мастни киселини. Приемът на ензимо-заместващия продукт подобрява чувствителността на рецепторите за LDL, с което намалява и съдържанието на мазнини в черния дроб и нормализира трансаминазите. Желателно е лечението да започне възможно най-скоро след поставяне на диагнозата. При деца с болест на Wolman (под 6 мес.) в зависимост от тежестта на състоянието и активността на LAL-ензима, дозата варира от 0.35mg - 3mg/kg за седмица. При деца с CESD дозата започва от 0.35mg - 1mg/kg за седмица, приложена чрез *венозна инфузия с продължителност 1-2 часа.* Многоцентрово двойно-сляпо плацебо-контролирано проучване (ARISE)[27] при 66 пациента с дефицит на LAL и провели терапия със Sebelipase alfa/Kanuma, отчита следните резултати след 3-месечен прием на медикамента: "нормализиране на стойностите на ALT с 31%, понижение на LDL-холестерола с 28% и подобрение на HDL-холестерола с 20% при таргетираната, спрямо плацебо контролирана група."[27]

Клиничен случай: Момче на 7 г. от първа бременност, протекла без оплаквания. Роден чрез секцио на термин. Нормален послеродов период. Проведени са всички

задължителни ваксинации. Оплакванията датират от 2 годишна възраст и се изявяват с: бавно прогресираща хепатомегалия, повишени серумни трансаминази, общ холестерол, LDL-холестерол и триглицериди.

На 2 г. детето е хоспитализирано и след проведени параклинични изследвания са отчетени завишени трансаминази ACAT/AST **73** U/l (0-34U/l) , АЛАТ/ALT **86** U/l (10-49U/l) и Холестерол **739** mmol/l. Ехографията на черен дроб е установила увеличени размери на черния дроб до 5 см по МКЛ, до 7 см по л. алба и леко уголемена слезка. Не са открити отклонения в допълнително назначените лабораторни, имунологични и хормонални изследвания. В следващите месеци стойностите на AST, ALT и LDL продължават да бъдат завишени и на 3 г. е осъществена чернодробна биопсия, данните от която насочват към болест на натрупването /"тезауризмоза"/. В диференциялно диагностичен план са изказани съмнения за фамилна хиперхолестеролемия и гликогеноза тип 9. Проведен е глюкагонов тест, който е в норма. Няма ясно становище за поставяне на окончателна диагноза. Препоръчан е диетичен режим на хранене.



На 6 год. възраст детето постъпва в УМБАЛ "Св. Марина". Налице е прогресиращо уголемяване на корема, повишени стойности на серумните трансаминази и холестерола. Теглото е 25.2 кг; ръст -118см. BMI - 17.8кг/м2 - на 75 персентил за пол и възраст. Настоящ ръст на 10-ти персентил, с намалена растежна скорост след 5 годишна възраст (фиг.1) и тегло на 50-ти персентил. Обиколката на корема е 60см. Артериално налягане е 85/50 mmHg, СЧ 86/мин. Детето е с адекватно умствено развитие. Черният дроб се палпира на 5 см в дясно под ребрена дъга и на 8 см по линия алба. Биохимичните показатели /Табл. 1/ отчетоха завишени стойности на ACAT - 90.0 U/l (0-34U/l), АЛАТ - 172U/l (10-49U/l), Холестерол - 9.29 mmol/l (2.70-5.20 mmol/l), LDL-C - 7.60 mmol/l (до 2.6 mmol/l), HDL-C 1.17 mmol/l (1.2-1.6 mmol/l). Не се откриха други отклонения в кръвната картина, както и в хормоналните изследвания. Ехографията на черен дроб показа умерена фиброза, хомогенна, хипоехогенна структура и размер на десен лоб: краниокаудален – 102 мм, АР - 95 мм и ляв лоб: АР – 44 мм. Проведената ехокардиография не откри миокардни и клапни нарушения. Фундоскопията на очни дъна установи леко нагънати периферни съдове с повишена рефлексогенност /Angiopathia retinae/. Към диагностичния план е назначена и костно-мозъчната пункция, резултатите от която показаха наличие на пенести клетки. При проведеното КТ изследване не се откри наличие на калцификати в надбъбреци.

Заключение.

Касае се за дете с клинични и лабораторни данни за хиперхолестеролемия. Наличната хепатомегалия с отлагане на масти в черния дроб, липсата на фамилна анамнеза за хиперхолестеролемия и данните от проведените по-рано чернодробна биопсия и костно-мозъчна пункция са в полза за наличие на ***хиперхлипидемия от тип 2б*** вследствие на натрупване на холестеринови естери. Резултатите от всички клинични и параклинични изследвания до момента, покриват голяма част от диагностичните

критерии публикувани от Željko Reiner et al[22] в полза на ***адултна форма на болест на Wolman (OMIM 278000)***. Диагнозата ни се потвърди и от проведения генетичен анализ в Rostock-Germany. Резултатът от него показа, че пациентът е смесен хетерозигот. Двете хетерозиготни мутации са идентифицирани на интрон 7 (het.c.822+1G>A) и екзон 8 (het.c.894G>A) от LIPA гена.

Проследяването на холестероловите и трансаминазни показатели през годините (табл1.), отчита плавното им покачване, без да има съществено влошаване в общото състояние на детето. Въвеждането на диетичен режим е довело до леко подобрение върху нивата на холестерола в сравнение с периода до 3-годишна възраст. Трансаминазите запазват тенденция към повишаване. Последните ехографски прегледи показват задълбочаване на хепатомегалията и фиброзирането на черния дроб. В предвид на отхвърлените ползи от терапията със статини се взе решение за започване на лечение със Sebelipase alfa. Стартирането на терапията изисква изследване на нивото на активност на LAL-ензима в организма. На базата на процента запазена активност се определя и началната терапевтична доза. Необходимо е редовното му проследяването и по време на терапията. За момента няма алтернатива за лечението на пацинети с адултна форма на болестта на Wolman.

Табл.1. Динамика с времето на биохимичните показатели при пациента.

	1г.	2г.	3г.	6г.	7г.
Холестерол	7.39 mmol/l	8.7 mmol/l	10.4 mmol/l	9.29 mmol/l	9,52 mmol/l
LDL-холестерол	-	7.4 mmol/l	8.31 mmol/l	7.60 mmol/l	7,70 mmol/l
HDL-холестерол	-	0.87 mmol/l	0.761 mmol/l	1.17 mmol/l	1,30 mmol/l
АСАТ	86 U/l	62 U/l	49 U/l	90.0 U/l	175 U/l
АЛАТ	73 U/l	76 U/l	58 U/l	172 U/l	119 U/l
ГГТ	19 U/l	19 U/l	19 U/l	25.0 U/l	28 U/l
CRP	37.97 mg/l	5.3 mg/l	-	5.18 mg/l	1,71 mg/l
Билирубин	7.7 umol/l	5.7mcmol/l	-	15.0 mcmol/l	16 mcmol/l

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Frequency of the Cholesteryl Ester Storage Disease Common *LIPA* E8SJM Mutation (c.894G>A) in Various Racial and Ethnic Groups

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Abstract

Cholesteryl Ester Storage Disease (CESD) and Wolman disease are autosomal recessive later-onset and severe infantile disorders, respectively, which result from the deficient activity of lysosomal acid lipase (LAL). LAL is encoded by *LIPA* (10q23.31) and the most common mutation associated with CESD is an exon 8 splice junction mutation (c.894G>A; E8SJM), which expresses only ~3–5% of normally spliced LAL. However, the frequency of c.894G>A is unknown in most populations. To estimate the prevalence of CESD in different populations, the frequencies of the c.894G>A mutation were determined in 10,000 *LIPA* alleles from healthy African-American, Asian, Caucasian, Hispanic and Ashkenazi Jewish individuals from the greater New York metropolitan area and 6,578 *LIPA* alleles from African-American, Caucasian, and Hispanic subjects enrolled in the Dallas Heart Study. The combined c.894G>A allele frequencies from the two cohorts ranged from 0.0005 (Asian) to 0.0017 (Caucasian and Hispanic), which translated to carrier frequencies of 1 in 1,000 to ~1 in 300, respectively. No African-American heterozygotes were detected. Additionally, by surveying the available literature, c.894G>A was estimated to account for 60% (95% CI: 51%–69%) of reported mutations among multi-ethnic

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CONFLICT OF INTEREST

R.J.D. is a consultant for and owns stock options of Synageva BioPharma, the company developing enzyme replacement therapy for LAL deficiency.

CESD patients. Using this estimate, the predicted prevalence of CESD in the Caucasian and Hispanic populations is ~0.8 per 100,000 (~1 in 130,000; 95% CI: ~1 in 90,000 to 1 in 170,000).

Conclusion—These data indicate that CESD may be under-diagnosed in the general Caucasian and Hispanic populations, which is important since clinical trials of enzyme replacement therapy for LAL deficiency are currently being developed. Moreover, future studies on CESD prevalence in African and Asian populations may require full-gene *LIPA* sequencing to determine heterozygote frequencies since c.894G>A is not common in these racial groups.

Keywords

lysosomal acid lipase deficiency; allele frequency; carrier frequency; disease prevalence; splice junction mutation; genotyping

INTRODUCTION

The deficient activity of lysosomal acid lipase (LAL), which hydrolyzes cholesteryl esters and triglycerides, results in either Cholesteryl Ester Storage Disease (CESD) or the more severe Wolman disease (1–2). LAL is encoded by the *LIPA* gene (10q23.31) (3) and homozygous and compound heterozygous *LIPA* mutations that result in little or no LAL activity cause Wolman disease, a severe infantile-onset disorder characterized biochemically by the massive storage of cholesteryl esters and triglycerides primarily in the liver, intestines, and adrenals leading to hepatosplenomegaly, adrenal calcification, vomiting, diarrhea, anemia, failure to thrive, and death typically before 1 year of age (4). In contrast, *LIPA* mutations with residual LAL activity result in CESD, which is characterized by hepatosteatosis, hepatomegaly, splenomegaly, dyslipidemia, accelerated atherosclerosis, and premature demise (1–2, 5–6).

Although over 40 *LIPA* mutations have been identified in patients with Wolman disease and CESD, the most common *LIPA* mutation is the c.894G>A exon 8 splice junction mutation (i.e., E8SJM; p.delS275_Q298; rs116928232). This mutation results in a transcript with an in-frame deletion of exon 8 that encodes a mutant enzyme with no residual LAL activity; however, the splicing defect also allows ~3–5% normally spliced LAL with enzyme activity (5, 7–9). Since the c.894G>A mutation results in residual LAL activity, it has only been found among patients with CESD and not Wolman disease (8, 10). In contrast, neighboring exon/intron 8 splice junction mutations, c.892C>T (E8SJM-3) and c.894+1G>A (E8SJM +1), are rare null alleles that have been detected in patients with Wolman disease. Interestingly, c.894G>A heterozygotes recently were found to have elevated total cholesterol compared to normolipidemic Caucasian controls, which may also impart an increased risk of coronary heart disease (CHD) (11). The association of *LIPA* as a susceptibility gene for CHD has been strengthened further by recent genome-wide association studies (12–14).

The frequency of CESD in different populations is unknown, but it is generally thought that the disease is often unrecognized. For example, it can be misdiagnosed in patients with hepatomegaly following a liver biopsy as non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or cryptogenic fatty liver disease (15). To estimate the prevalence of CESD, a previous study investigated the frequency of the c.894G>A CESD mutation in the German population and identified an allele frequency of 0.0025 (1 in 202 carrier frequency) (16). Among the reported CESD patients who had undergone *LIPA* mutation analyses, c.894G>A previously was estimated to account for ~50% of all CESD alleles (16). As such, with this assumption, the identified 0.0025 c.894G>A allele frequency predicted a CESD prevalence of ~2.4 per 100,000 in the German population.

Since no *LIPA* c.894G>A allele frequencies exist for other racial or ethnic groups, including the African-American, Asian or Hispanic populations, efforts were directed to determine the c.894G>A frequencies in individuals from five major racial and ethnic groups from both the New York metropolitan area and the Dallas Heart Study. The frequency of the c.894G>A mutation in various populations and its prevalence among CESD patients can be used to estimate the prevalence of CESD, which may encourage hepatologists, lipidologists, pathologists, geneticists, and metabolic physicians to identify CESD patients since clinical trials of enzyme replacement therapy for LAL deficiency are underway and may provide safe and effective therapy.

MATERIALS AND METHODS

c.894G>A Prevalence in CESD

The prevalence of the c.894G>A mutation among unrelated multi-ethnic CESD patients was estimated by searching available literature and CESD case reports. The PubMed database (NCBI) was searched using the keywords (CESD OR Cholesteryl Ester Storage Disease) AND (mutation) from 1966 to September 2012. Studies were considered for inclusion if *LIPA* mutation status was reported for unique patient(s) with clinically confirmed CESD. Only a single affected patient was included in the *LIPA* mutation prevalence calculation when reports included multiple affected family members with the same mutations. Additionally, the Human Gene Mutation Database (HGMD) Professional (http://www.biobase-international.com/product/hgmd) was interrogated for CESD-causing *LIPA* mutations.

Study Population

New York Metropolitan Area—Peripheral blood samples from healthy donors who indicated their racial/ethnic background and gave informed consent for the use of their DNA for research were obtained from the New York Blood Center with Institutional Review Board (IRB) approval (17–18). In addition, blood samples were obtained with informed consent from unrelated, healthy 100% Ashkenazi Jewish (AJ) individuals from the greater New York metropolitan area as previously defined (19–20). Allpersonal identifiers were removed, and isolated DNA samples were tested anonymously. Genomic DNA was isolated using the Puregene® DNA Purification kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. For this study, 1,000 individuals each from five different populations (African-American, Asian, Caucasian, Hispanic, and AJ) were subjected to c. 894G>A genotyping.

Dallas Heart Study—The Dallas Heart Study is a multi-ethnic population-based probability sample of Dallas County residents (ages 18–85), weighted to include approximately 50% of African-American subjects. Sampling design and recruitment procedures have been previously described in detail (21). The study was transformed from a cross-sectional study to a longitudinal study in 2007 (DHS-2) when all prior DHS participants were invited to the clinic for a repeat evaluation. Race or ethnic identity was self-reported according to a list of categories used in the United States census. A total of 3,401 individuals submitted blood samples and provided written consent for genetic analyses. For this study, 1,720 African-American, 1,089 Caucasian, and 480 Hispanic participants from the DHS-2 cohort were subjected to c.894G>A genotyping. The study was approved by the IRB of the University of Texas Southwestern Medical Center at Dallas.

Genotyping

LIPA c.894G>A genotyping was performed using custom-designed TaqMan® allelic discrimination assays (Applied Biosystems, Carlsbad, CA) as described in the Supplemental

Data. Selected mutation carriers were confirmed by bidirectional sequencing with independent M13-tagged *LIPA* amplification primers (forward: 5′-TGCTTTGAAGGGCAAAATAC-3′; reverse: 5′-TTTCTATTTGGAAAGGGTTTGC-3′) and analyzed using Mutation Surveyor software v3.30 (SoftGenetics).

CESD Prevalence

Prevalence of CESD was calculated using identified c.894G>A allele frequencies and adjusting for the prevalence of c.894G>A among CESD patients, as previously reported (16).

RESULTS

Prevalence of the c.894G>A Mutation in CESD

To estimate the prevalence of the c.894G>A mutation among unrelated multi-ethnic patients with CESD, a literature survey was conducted which revealed that c.894G>A accounted for 60% (95% CI: 51%–69%) of reported multi-ethnic CESD mutations (n=55 patients) (Table 1). These results are consistent with the previous c.894G>A frequency estimate of ~50% among CESD patients (16).

c.894G>A Allele and Carrier Frequencies

The identified c.894G>A allele and carrier frequencies are summarized in Table 2 and a representative sequence chromatogram of a confirmed heterozygote is illustrated in Figure 1. The c.894G>A allele frequencies from the New York metropolitan area cohort ranged from 0.0005 (Asian) to 0.0015 (Caucasian and Hispanic), which translated to carrier frequencies of 1 in 1,000 to 1 in 333, respectively. The c.894G>A allele frequencies from the Dallas Heart Study cohort ranged from 0.0018 (Caucasian) to 0.0021 (Hispanic), which translated to carrier frequencies of 1 in 272 to 1 in 240, respectively. No African-American carriers were detected in either cohort. Additionally, the Caucasian and Hispanic c.894G>A frequency data from the New York and Dallas cohorts were combined, which resulted in similar allele frequencies of 0.0017 and carrier frequencies of 1 in 298 and 1 in 296, respectively. Of note, with a c.894G>A carrier frequency of ~1 in 300 and a detectability of only 0.60, Bayesian analysis indicates that the residual risk of being a CESD carrier after negative c.894G>A carrier screening in the Caucasian and Hispanic populations would be ~1 in 500.

Predicted Prevalence of CESD

The predicted prevalence of affected individuals with CESD in the different populations, assuming Hardy-Weinberg equilibrium, is also summarized in Table 2. CESD prevalence was calculated using the identified c.894G>A allele frequencies and adjusting for the prevalence of c.894G>A among CESD patients. Assuming that the c.894G>A mutation accounts for 60% (95% CI: 51%–69%) of known multi-ethnic CESD mutations, the predicted prevalence of CESD in the Caucasian population of the greater New York metropolitan area is ~0.6 per 100,000 (1 in 160,000; 95% CI: 1 in 114,898 to 1 in 212,553) and ~0.9 per 100,000 among Caucasians from the Dallas Heart Study (1 in 106,733; 95% CI: 1 in 76,646 to 1 in 141,790). When combined, the prevalence in the general Caucasian population was determined to be ~0.8 per 100,000 (1 in 128,246; 95% CI: 1 in 92,095 to 1 in 170,369). For the Hispanic population, CESD prevalence was estimated at ~0.6 in 100,000 and ~1.2 in 100,000 in the New York and Dallas cohorts, respectively, and ~0.8 in 100,000 (1 in 126,167; 95% CI: 1 in 90,602 to 1 in 167,607) in the combined Hispanic population.

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Clinical Effect and Safety Profile of Recombinant Human Lysosomal Acid Lipase in Patients with Cholesteryl Ester Storage Disease

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Abstract

Background & Aims—Cholesteryl Ester Storage Disease, an inherited deficiency of lysosomal acid lipase, is an underappreciated cause of progressive liver disease with no approved therapy. Presenting features include dyslipidemia, elevated transaminases, and hepatomegaly.

Methods—To assess the clinical effects and safety of the recombinant human lysosomal acid lipase, sebelipase alfa, 9 patients received 4 once-weekly infusions (0.35, 1, or 3 $mg \cdot kg^{-1}$) in LAL-CL01 which is the first human study of this investigational agent. Patients completing LAL-CL01 were eligible to enroll in the extension study (LAL-CL04) in which they again received 4 once-weekly infusions of sebelipase alfa (0.35, 1, or 3 $mg \cdot kg^{-1}$) before transitioning to long term every other week infusions (1 or 3 $mg \cdot kg^{-1}$).

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Results—Sebelipase alfa was well-tolerated with mostly mild adverse events unrelated sebelipase alfa. No anti-drug antibodies were detected. Transaminases decreased in patients in LAL-CL01 and increased between studies. In 7 patients receiving ongoing sebelipase alfa treatment in LAL-CL04, mean±SD decreases for alanine transaminase and aspartate aminotransferase at week 12 compared to the baseline values in LAL-CL01 were 46±21U/L (-52%) and 21±14U/L (-36%), respectively ($p<0.05$). Through week 12 of LAL-CL04, these 7 patients also showed mean decreases from baseline in total cholesterol of 44±41mg/dL (-22%; p=0.047), low density lipoprotein-cholesterol of 29±31mg/dL (-27%; p=0.078), and triglycerides of 50±38mg/dL (-28%, p=0.016) and increases in high density lipoprotein-cholesterol of 5mg/dL (15%; p=0.016).

Conclusions—These data establish that sebelipase alfa, an investigational enzyme replacement, in patients with Cholesteryl Ester Storage Disease is well tolerated, rapidly decreases serum transaminases and that these improvements are sustained with long term dosing and are accompanied by improvements in serum lipid profile.

Keywords

lysosomal storage; enzyme replacement; fatty liver; hepatomegaly; dyslipidemia

Lysosomal Acid Lipase (LAL) Deficiency [(LAL Deficiency) OMIMD 278000] is a multisystem autosomal recessive disease caused by mutations in the *LIPA* gene, which encodes the enzyme, lysosomal acid lipase. LAL Deficiency leads to the accumulation of cholesteryl esters and triglycerides in the lysosomes of many tissues, including the liver, spleen, and cardiovascular system (1). LAL Deficiency presents as a clinical continuum with two major phenotypes: a rapidly progressive form, frequently called Wolman Disease, which manifests in infants, and a form that manifests post-infancy, also called Cholesteryl Ester Storage Disease (CESD). CESD is an under-appreciated cause of fatty liver, with prominent microvesicular steatosis, hepatic fibrosis and progression to cirrhosis and early death. Although the natural history of the disease has not been well studied, serious liver complications are frequently described. Splenomegaly and cardiovascular involvement are also commonly seen. Cardiovascular involvement includes accelerated (2) and premature (3) atherosclerosis associated with dyslipidemia (high total and low density lipoprotein-cholesterol [LDL], high triglyceride, and low high density lipoprotein-cholesterol [HDL]). The management of patients with CESD has mainly focused on control of the dyslipidemia through diet and the use of lipid lowering therapies including statins (4-10). Although laboratory improvements may be seen in some cases (4, 6-10), the underlying disease persists and disease progression still occurs (5, 11).

While the potential for enzyme replacement therapy as a treatment for patients with LAL Deficiency has been recognized for more than 25 years (12, 13), earlier attempts to produce recombinant LAL using different manufacturing approaches (Chinese Hamster Ovary (14), yeast (14), and plant-based production systems (15)) did not yield a therapeutic enzyme that progressed into clinical development.

Sebelipase alfa (SBC-102; Synageva BioPharma Corporation, Lexington, Massachusetts, USA) is a recombinant human LAL produced using methodologies that allow targeted expression of a gene sequence (16) in hen oviduct cells (17). The expressed gene sequence encodes for the same amino acid sequence as the native human LAL enzyme with secretion of the recombinant protein into egg white. Sebelipase alfa is the International Nonproprietary Name given to SBC-102 in 2012. In a rat model of LAL Deficiency that replicates a number of the abnormalities seen in patients with the disease (18, 19), sebelipase alfa produced a dose-dependent decrease in transaminases, improvement in liver pathology, and correction of impaired weight gain (20).

This is the first clinical report of the use of sebelipase alfa in patients with liver abnormalities due to CESD. The initial clinical trial and the long term treatment study were designed to characterize the safety, pharmacokinetics, and pharmacodynamic activity of repeat dosing with sebelipase alfa. The pharmacokinetic profile will be reported separately.

Patients and Methods

Sebelipase alfa

Sebelipase alfa is a glycoprotein with six potential N-linked glycosylation sites, of which five are occupied. Structural and compositional analyses demonstrate that sebelipase alfa glycans consist of predominantly N-acetylglucosamine and mannose terminated N-linked structures, which target proteins to the mannose receptors. N-glycans containing terminal mannose-6-phosphate moieties are also consistently expressed in sebelipase alfa, which allow for targeting to a wide variety of cells expressing the mannose-6-phosphate receptor. Cell culture studies (17) show that sebelipase alfa demonstrates mannose receptor dependent uptake and traffics to the lysosomes in a rat macrophage cell line. Additionally sebelipase alfa demonstrates mannose-6-phosphate receptor-dependent uptake and corrects the intracellular enzyme deficiency in fibroblasts from a LAL deficient patient. The specific activity of sebelipase alfa is approximately 260 U/mg protein [one unit is defined as the amount of activity that results in the hydrolysis of 1 μmole of a synthetic substrate, 4-methylumbelliferyl oleate, per minute under the assay conditions] (data on file, Synageva BioPharma Corporation).

Study design

A Phase 1/2 open-label, multicenter, dose-escalation study (LAL-CL01) was conducted between April 2011 and January 2012 across 6 sites in 4 countries. Patients completing LAL-CL01 were eligible to enroll in the LAL-CL04 extension study to further evaluate the clinical effects of sebelipase alfa. The research was conducted in accordance with the Declaration of Helsinki and Good Clinical practice guidelines. The protocols were approved by the appropriate ethics committees or institutional review boards at participating institutions and conducted in compliance with country specific regulatory requirements. The trials were registered as NCT01307098: CL01 (http://www.clinicaltrials.gov/ct2/show/NCT01307098) and NCT01488097: CL04 (http://www.clinicaltrials.gov/ct2/show/NCT01488097). All patients provided informed written consent before undergoing study-specific assessments or procedures.

In LAL-CL01, screening assessments were conducted 7 to 28 days prior to the start of dosing. Eligible patients were treated in 3 sequential dose cohorts (Figure 1): 0.35 $mg \cdot kg^{-1}$ (low dose, Cohort 1: patients 1-3), 1 $mg \cdot kg^{-1}$ (intermediate dose, Cohort 2: patients 4-6), or 3 $mg \cdot kg^{-1}$ (high dose, Cohort 3: patients 7-9). In each cohort, patients were administered once-weekly infusions of sebelipase alfa on Day 0, Day 7, Day 14, and Day 21 with monitoring for safety. Dose escalation to 1 $mg \cdot kg^{-1}$ and 3 $mg \cdot kg^{-1}$ dose levels was based on review of safety data by an independent Safety Committee. Following completion of the last infusion on Day 21, patients returned on Day 28 and Day 52 for assessment of safety and the magnitude and reversibility of effects on serum transaminases, lipids, and other markers of disease activity. Throughout the study, patients on lipid lowering medications continued on their medications at a stable dose. Sebelipase alfa was provided as a liquid solution in single use glass vials and diluted for infusion in normal saline. Diluted sebelipase alfa was administered intravenously over a period of approximately 120 minutes.

After completion of LAL-CL01, patients were eligible to enroll in the LAL-CL04 extension trial to evaluate the long-term effects of sebelipase alfa (Figure 1). In this study, patients

resumed treatment with 4 once-weekly infusions of sebelipase alfa at the same dose as in LAL-CL01 study (0.35, 1.0 or 3.0 $mg \cdot kg^{-1}$) before transitioning to every other week infusions (1.0 or 3.0 $mg \cdot kg^{-1}$). Patients in the once-weekly 0.35 $mg \cdot kg^{-1}$ dosing regimen moved to 1 $mg \cdot kg^{-1}$ every other week. Patients in the 1 and 3 $mg \cdot kg^{-1}$ once-weekly dosing cohorts remained on the same dose but received it every other week.

Patients

Eligible patients were 18 to 65 years old. Deficient enzyme activity was confirmed upon entry into LAL-CL01 at our central laboratory (Willink Biochemical Genetics Unit Regional Genetics Laboratory, Manchester, England). Additionally, patients were required to have either hepatomegaly on physical exam or elevated transaminases (between 1.5 and 3 times the upper limit of normal of the clinical lab). Patients receiving statins or ezetimibe were only eligible if on a stable dose for at least 4 weeks prior to screening. Female patients of child-bearing potential and male partners of potentially fertile women were required to use a medically acceptable form of contraception. Patients were excluded from this study if they had severe hepatic dysfunction (Child-Pugh Class C); aspartate aminotransferase (AST) and/or alanine transaminase (ALT) persistently elevated to greater than three times the upper limit of normal; chronic liver disease attributed to a cause other than CESD; serological evidence of hepatitis B virus or hepatitis C virus; a score of 8 or more on a screening Alcohol Use Disorders Identification Test; previous hematopoietic bone marrow or liver transplant; known hypersensitivity to eggs; had received an investigational therapy for another indication within 30 days of screening; or they could not comply with the protocol for any other reasons.

Safety Assessments

Patients were monitored for safety and tolerability of sebelipase alfa at regular intervals from the time of signing the informed consent. In LAL-CL01, these assessments continued until the end of study assessment on Day 52. In the ongoing LAL-CL04 extension study, these assessments are analyzed to week 12. Safety evaluations included monitoring of adverse events, concomitant medications, physical examinations, vital signs, and clinical laboratory tests.

Pharmacodynamic assessments

Biological activity of sebelipase alfa was assessed by analysis of hepatic transaminases (ALT, AST), lipid parameters (total cholesterol, triglycerides, HDL, LDL), and serum ferritin. These were selected based on insights from preclinical studies of sebelipase alfa (20, 21) and disease biology (1). Ferritin was analyzed because it is increased in the presence of macrophage activation (22) and has been reported to be elevated in patients with LAL Deficiency (23).

Anti-drug Antibody assays

The presence of serum anti-sebelipase alfa antibodies was examined by use of a validated (24, 25) "bridging ELISA." In these assays, sebelipase alfa was immobilized onto 96-well microtiter plates. Serum samples were subsequently added to the plates followed in series by biotinylated sebelipase alfa, streptavidin-peroxidase, and TMB substrate. This was used as the "screening assay" with a positivity cut-point predetermined by analysis of normal human serum samples. Putative positive samples were subjected to confirmatory immunodepletion assays in which sebelipase alfa was spiked into the serum samples, before the samples were evaluated using the same bridging assay. This assay has a sensitivity of ≥312 ng/mL in serum and has been used previously to successfully detect anti-sebelipase alfa antibodies in pre-clinical studies in rats and cynomolgus monkeys (data on file, Synageva Biopharma).

Statistical analysis

All patients who received at least 1 dose of sebelipase alfa were included in the safety analysis. Adverse events, vital signs, and laboratory tests including pharmacodynamic assessments and pharmacokinetics, were summarized. The study was not powered to detect differences between cohorts and therefore no statistical comparisons of cohorts were performed. Exploratory statistical analyses were performed to examine the effects of sebelipase alfa on key activity parameters in both LAL-CL01 and LAL-CL04. Wilcoxon's sign-rank test was used for statistical tests of change from baseline, without adjustment for multiplicity.

Results

Nine patients met study eligibility criteria and were sequentially enrolled to one of the three dose cohorts; all 9 patients received four weekly infusions in the LAL-CL01 study. At the time of this analysis, seven of these patients have received sebelipase alfa through Week 12 in the LAL-CL04 study. In these 7 patients, the median (range) wash-out period off between the last dose of sebelipase alfa in LAL-CL01 and the first dose of sebelipase alfa in LAL-CL04 was 15.1 weeks (range 9.0 to 28.3 weeks).

Demographics and baseline disease characteristics

Baseline characteristics of the study population are described in Table 1. All patients were Caucasian and two-thirds were male, with a mean age of 31.6 ± 10.7 years (range 19 to 45 years) at the time of enrollment. Dose cohorts were similar with respect to the patients' age and gender. Mean body mass index was 26.8 (SD ± 6.3; range 20.5 to 42.4). Medical history findings were consistent with those expected in this patient population. Seven patients had a history of hepatomegaly and/or splenomegaly, and 2 patients had evidence of more advanced liver disease; one subject in Cohort 2, had cirrhosis and portal hypertension and one subject in Cohort 3 had periportal fibrosis. Eight of the 9 patients had clinical evidence of hepatomegaly on physical exam. Seven (77.8%) patients also had a history of other cardiovascular conditions. Seven (77.8%) patients were on one or more medications at enrollment, and all 7 were receiving treatment with lipid-modifying therapies, including ezetimibe, statins, and other medications. No change in lipid modifying therapies was made during the studies. One patient (#3) discontinued lipid lowering medication between completion of LAL-CL01 and beginning LAL-CL04.

Safety

LAL-CL01—All 9 patients completed LAL-CL01 with no serious adverse events, treatment-related discontinuations, withdrawals or dose reductions. A total of 36 infusions of sebelipase alfa were administered to the 9 patients with no infusion-related reactions, no requirement for premedication with antipyretics or antihistamines, or modification of infusion rate due to infusion related side effects. No anti-sebelipase alfa antibodies were detected in any subject.

Seven of the nine patients experienced adverse events. Nausea, headache and diarrhea were reported in 2 or more patients (Table 2). The overall frequency of adverse events was comparable for the two highest dose cohorts and less frequent in Cohort 1. The majority of events were mild (grade 1) and only six events in 2 patients were considered possibly related to study drug. One subject in Cohort 3 developed asymptomatic elevation in cholesterol (total cholesterol increased from 220 mg/dL at baseline to 772 mg/dL at Day 28), which was classified as a grade 4 event based on the National Cancer Institute Common Terminology Criteria adverse event grading scale used in this study (ctep.cancer.gov/reporting/ctc.html). One additional subject in Cohort 1 had an asymptomatic increase in cholesterol to over 400

mg/dL, from 391 mg/dL at baseline to 509 mg/dL at Day 28, which was not considered an adverse event by the investigator. There were no clinically relevant trends in systolic or diastolic blood pressure, heart rate, respiratory rate, or body temperature during this study.

LAL-CL04—At the time of this report, 7 of the 9 patients have transitioned into the ongoing extension study and have been administered a total of 56 infusions over the 12 week period. With the exception of occasional episodes of mild diarrhea temporally associated with some infusions in 2 patients, the overall safety profile through 12 weeks in LAL-CL04 remains consistent with that in LAL-CL01 with no findings of clinical concern. A single patient during LAL-CL04 experienced acute cholecystitis classified as a serious adverse event but deemed by the investigator unlikely to be related to sebelipase alfa. All 7 patients remained negative for anti-sebelipase alfa antibodies through the 12 week visit.

Pharmacodynamic Activity

Liver transaminases—Following initiation of treatment with sebelipase alfa, levels of ALT and AST decreased rapidly in 8 of the 9 patients, regardless of whether their baseline levels were within or above the normal range. No obvious difference in response was seen between cohorts, and therefore data were pooled for all patients. This reduction in AST and ALT was apparent within 2 weeks of the first infusion and levels continued to decrease in most patients through Day 28. By Day 28, approximately 1 week after the fourth infusion, transaminases had normalized in all 6 patients with abnormal baseline ALT and in 4 (66.7%) of 6 patients with abnormal baseline AST. For the set of all 9 patients, treatment was associated with statistically significant decreases ($p<0.05$) in AST and ALT from baseline to Day 28. The mean decreases from baseline to Day 28 were 39 ± 26 U/L (41% decrease) for ALT and 18 ± 15 U/L (32% decrease) for AST (Figure 2). As shown in Figure 2, the mean improvements in ALT and AST were sustained for two weeks after the last infusion but had partially reversed 3 weeks later. There was no evidence of a dose-related effect in the time to onset or magnitude of the reduction in AST and ALT, or in the maintenance of that effect after discontinuation of treatment.

Transaminases, which decreased with therapy in LAL-CL01, increased in patients off treatment. All 7 patients who re-initiated sebelipase alfa in LAL-CL04 had rapid reductions in transaminases similar to those seen in LAL-CL01, which were sustained with the transition to every other week dosing. In the 7 patients receiving ongoing treatment with sebelipase alfa in LAL-CL04, the statistically significant ($p<0.05$) decreases at week 12 for ALT and AST (shown as mean decrease ± SD compared to the baseline values in LAL-CL01) were 46 ± 21 U/L (52% decrease) and 21 ± 14 U/L (36% decrease), respectively (Figure 2).

In the one patient with a markedly elevated gamma-glutamyl transpeptidase of 203 U/L at baseline in LAL-CL01, gamma-glutamyl transpeptidase declined to 96 U/L at Day 28 before increasing to 187 U/L off treatment between studies. Upon reinitiating treatment in LAL-CL04, gamma-glutamyl transpeptidase decreased to 64 U/L at week 12.

Serum lipids—Total cholesterol, triglycerides, and LDL increased in most patients between baseline and Day 28. Increases in total cholesterol and triglycerides were observed for 8 patients with 7 of these patients also had increases in LDL. On average, total cholesterol increased by 140 ± 168 mg/dL (70%), triglycerides increased by 82 ± 91 mg/dL (69%), and LDL increased by 113 ± 169 mg/dL (87%) between baseline and Day 28. The magnitudes of the increases in these serum lipids were comparable in the two lowest dose cohorts, and more pronounced in Cohort 3. While one subject in Cohort 3 showed changes comparable to those seen in the 6 patients in Cohorts 1 and 2, the largest increase in total

cholesterol, LDL, or triglycerides were seen in the other two patients in the high dose cohort. Post treatment lipid monitoring was not performed for patients in Cohort 1. By Day 52, thirty-one days after the last dose of sebelipase alfa in LAL-CL01, 5 of the 6 patients in Cohorts 2 and 3 had levels for each lipid parameter that were below their baseline values. One patient had normal total cholesterol and triglycerides, and a borderline abnormal LDL that was approaching baseline levels. HDL levels were generally stable during treatment with sebelipase alfa in LAL-CL01, with no consistent changes between baseline and Day 28 (range -8 to +9 mg/dL).

In the 7 patients receiving ongoing treatment with sebelipase alfa in LAL-CL04 through Week 12, all 7 patients showed decreases from their original LAL-CL01 baseline values in triglycerides ($p=0.016$) and increases in HDL ($p=0.016$); 6 of 7 patients had decreases in total cholesterol ($p=0.047$) and LDL ($p=0.078$). The mean ±SD decreases for total cholesterol, LDL, and triglycerides were 44±41 mg/dL (-22%), 29 ± 31 mg/dL (-27%), and 50 ± 38 mg/dL (-28%), respectively. Mean HDL increased from 35 ± 9 mg/dL to 40 ± 9 mg/dL (15%)(Figure 3). The one patient who did not have a decrease in total cholesterol and LDL had discontinued lipid lowering therapy between completing LAL-CL01 and beginning LAL-CL04.

In addition to effects on liver transaminases and serum lipids, serum ferritin decreased in all 9 patients between baseline and Day 28. Mean decreases in serum ferritin from baseline to Day 28 were comparable for Cohort 2 (112 ± 34 ng/mL) and Cohort 3 (119 ± 69 ng/mL), and appeared to be slightly greater than those for Cohort 1 (54± 11 ng/mL). Ferritins measured at the beginning of LAL-CL04 were higher than the last follow-up measurements at the conclusion of LAL-CL01 in 5 of 7 patients. Nonetheless, by week 12 of treatment in LAL-CL04, there was a decrease in serum ferritin in all 7 patients compared to both the baseline values in LAL-CL01 and the baseline values in LAL-CL04. Mean changes by gender for these 7 patients are shown in Figure 4.

Discussion

Herein we report on the first–in-human experience of enzyme replacement therapy with the recombinant human lysosomal acid lipase enzyme, sebelipase alfa, in CESD patients. Diagnosis of CESD requires a high index of clinical suspicion as the combination of fatty liver, elevated transaminases and dyslipidemia is also seen in patients with the much more common diagnosis of metabolic syndrome. As is the case for some other lysosomal storage diseases (26), substrate accumulation in LAL Deficiency is also associated with macrophage activation, elevation of ferritin (23), and chitotriosidase (27). The results of these studies demonstrate that sebelipase alfa is well tolerated with infrequent, mild reactions. The majority of adverse events were mild and unrelated to study drug. Taken together, this patient population has now received more than 90 infusions with no infusion-related allergic or hypersensitivity-type reactions. Our initial clinical experience suggests that sebelipase alfa may be less immunogenic than many other enzyme replacement therapies. This decreased immunogenicity may be due to (A) the rapid clearance of sebelipase alfa from the circulation following administration (data not shown), (B) the ubiquitous expression of homologous lipases that may decrease the likelihood that administered sebelipase alfa will represent a "foreign" antigen likely to induce an immune response even in patients where the enzyme is completely absent. (28), and (C) the finding that these patients, as expected in patients with CESD, do have some residual LAL enzyme activity (Table 1).

While the main objective of these studies was to evaluate the safety of sebelipase alfa, they provide evidence that enzyme replacement therapy with sebelipase alfa in patients with CESD is mobilizing accumulated lysosomal lipid, and that this is accompanied by

normalization of serum transaminases and improvements in the serum lipid profile. Additionally, there was evidence that substrate mobilization is associated with rapid reductions in ferritin which suggests that correction of enzyme deficiency also is accompanied by a reduction in macrophage activation. The significant reductions in ALT, AST and ferritin after the commencement of enzyme replacement therapy with sebelipase alfa were rapid and sustained. The time course of the reversibility of the effects of sebelipase alfa on these pharmacodynamic biomarkers after cessation of dosing following LAL-CL01 supports consideration of either weekly or every other week dosing.

Characteristically, some patients demonstrated elevated serum total cholesterol, LDL, and/or triglycerides, despite having been on various regimens of lipid lowering agents (5). Increases in total cholesterol, triglyceride and LDL were seen in all cohorts after 4 weekly infusions of sebelipase alfa in LAL-CL01, which rapidly reversed following discontinuation of therapy. In most patients, serum cholesterol, triglyceride and/or LDL were below their baseline values at Day 52. The transient rise in serum total cholesterol, LDL, and triglyceride following initiation of treatment with sebelipase alfa may be the result of increased free cholesterol and fatty acids in the cytoplasm leading to increased secretion of VLDL and down-regulation of the LDL receptor. The finding that there was not a concomitant rise in either alkaline phosphatase or gamma-glutamyl transpeptidase suggests that increases in cytoplasmic free fatty acids and cholesterol are not accompanied by transient intrahepatic cholestasis (supplemental Figure 1). More importantly, with continued treatment with sebelipase alfa in LAL-CL04, the mean serum total cholesterol, LDL, and triglycerides all decreased to below original baseline levels at week 12. This trend strongly suggests that continued sebelipase alfa treatment is correcting the dyslipidemia associated with enzyme deficiency in addition to its effects on mobilizing accumulated lipid from the liver, spleen, and other tissues.

Decreased HDL levels have also been described in patients with CESD and a recent study has established a link between LAL activity, cholesteryl ester, and triglyceride breakdown products and expression of the ABCA1 transporter which is important for reverse cholesterol transport (29). With the more sustained dosing in LAL-CL04, HDL levels significantly increased by week 12 compared to baseline in LAL-CL01. With enzyme replacement, the decreases seen in LDL and triglycerides, together with the increases seen in HDL, are important given the recognized clinical sequelae of premature atherosclerosis reported in CESD (1, 2).

In summary, these initial human clinical studies demonstrate that sebelipase alfa is well tolerated with an acceptable safety profile over a broad range of doses in this CESD patient population. ,. We have established that enzyme replacement produces a rapid and sustained reduction in transaminases in patients with CESD. Consistent with the hypothesis that sebelipase alfa can rapidly mobilize abnormal lysosomal lipid from affected tissues in these patients, treatment was associated with rapid but transient increases in serum total cholesterol, LDL, and triglycerides. As anticipated from the recognized association between CESD and dyslipidemia, continued treatment with enzyme replacement led to statistically significant decreases to below baseline levels in not only serum total cholesterol and triglycerides, downward trends in LDL, and but also modest but significant improvements in HDL. Taken together, these findings provide evidence that sebelipase alfa corrects a broad range of abnormalities associated with this inherited enzyme deficiency and has the potential to improve the clinical course for patients afflicted with CESD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

SPECIAL REPORT

Hypercholesterolaemia and hepatosplenomegaly: Two manifestations of cholesteryl ester storage disease

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ABSTRACT

Cholesteryl ester storage disease (CESD) is a rare autosomal recessive disease caused by mutations in *LIPA*. Here we describe two different clinical presentations of this disease: one case with a clear phenotype of familial hypercholesterolaemia and one case with hepatosplenomegaly from childhood onwards. These two cases exemplify the diversity of clinical phenotypes of patients with CESD. Knowledge on the phenotypic variability of the disease is of clinical relevance in light of enzyme replacement therapy (sebelipase alpha) for patients with mutations in *LIPA*, which is currently under development.

What was known on this topic?
Cholesteryl ester storage disease is a rare lysosomal storage disease caused by mutations in the *LIPA* gene.

What does this add?
We show that the variability of phenotypes of patients with CESD is very large and illustrate that in patients with hypercholesterolaemia of unknown origin or unexplained hepatosplenomegaly, *LIPA* mutations could be present.

KEYWORDS

Cholesteryl ester storage disease, lysosomal acid lipase, familial hypercholesterolaemia, low-density lipoprotein cholesterol

INTRODUCTION

Cholesteryl ester storage disease (CESD), or lysosomal acid lipase deficiency (MIM #613497), is an autosomal recessive disease caused by mutations in the *LIPA* gene, encoding lysosomal acid lipase (LAL). LAL hydrolyses cholesteryl esters and triglycerides in lysosomes and a deficiency of this enzyme leads to intracellular accumulation of cholesteryl esters and triglycerides in hepatocytes, adrenal glands, intestine and cells of the macrophage-monocyte system.[1] As a consequence, patients suffering from LAL deficiency are in general characterised by hepatomegaly, splenomegaly, diarrhoea and mixed hyperlipidaemia. Depending on the residual enzyme activity, LAL deficiency either results in the very severe and lethal Wolman disease (< 12 months of life) or the late-onset form, called CESD.[1] CESD often remains undiagnosed until severe symptoms (i.e. liver failure and/or atherosclerotic disease) occur in adulthood.[2] To date, ~140 CESD patients have been reported in literature while the prevalence of the disease has been estimated to vary between 1:40,000 and 1:400,000 individuals.[2-4] The discrepancy in the reported and estimated number of patients might be caused by a large phenotypical variation of the disease and unawareness among medical professionals. We here present two family cases with substantially different clinical presentations of CESD, illustrating that this disease might be easily missed or remain undiagnosed.

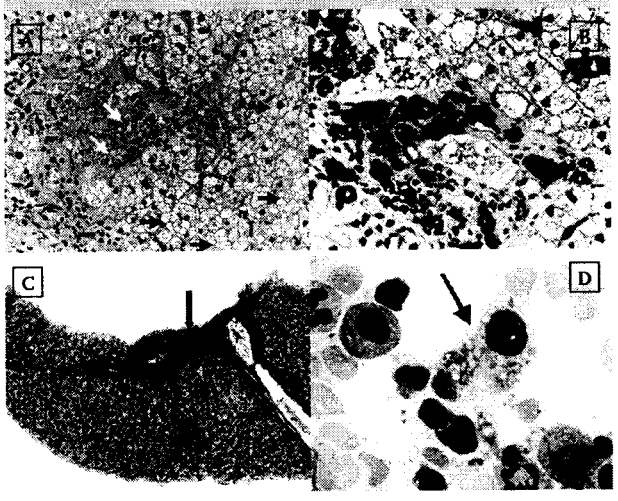
CASE 1

A 23-year-old female was diagnosed with a clinical phenotype of primary hypercholesterolaemia during a routine cholesterol screening at work (total cholesterol 13.1 mmol/l; low-density lipoprotein-cholesterol (LDL-C 10.6 mmol/l; high-density lipoprotein-cholesterol (HDL-C) 1.75 mmol/l; triglycerides 1.69 mmol/l). She did not have any symptoms and physical examination was normal except for a systolic cardiac murmur (grade IV/VI) due to a supravalvular aortic stenosis. Subsequent laboratory analysis only revealed mild increases in alanine aminotransferase (ALAT) levels in the proband (56 U/l; reference: 0-45 U/l).[4] Family screening showed that both of her siblings were also affected while lipid levels were normal in their parents.[4] However, no mutation was identified in one of the well-annotated genes for either autosomal dominant or recessive hypercholesterolaemia, (*LDLR*, *APOB*, *PCSK9* and *LDLRAP*). In order to find a mutation in an unknown gene as cause of the hypercholesterolaemic phenotype, we sequenced all protein coding regions of the genome (exome sequencing). Unexpectedly, homozygosity for the exon 8 splice junction (E8SJ = c.G934A) mutation in *LIPA* was found.[5] The patients were therefore diagnosed with CESD which was biochemically confirmed by a residual LAL activity of 8% in the proband.[4] Both siblings of the proband were also found to be homozygous for the E8SJ mutation and all three patients were treated with statin therapy resulting in an impressive decrease in LDL-C levels by 73 to 88% in the proband and her siblings.[4] We performed magnetic resonance spectroscopy in order to determine the consequences of the *LIPA* mutations on cholesteryl ester storage accumulation in the liver. Both the proband and her two siblings turned out to have substantial hepatic cholesteryl ester accumulation, but hepatosplenomegaly was not observed.[4] This case was previously published by Stitziel and co-workers.[4]

CASE 2

A 34-year-old male of Dutch/Indonesian descent was admitted to the hospital because of upper abdominal pain, diarrhoea and intestinal blood loss. His past medical history was relevant for hepatosplenomegaly of unknown origin since childhood, for which no follow-up had taken place. A surgical biopsy obtained when he was 3 years old remained inconclusive. Hepatosplenomegaly without signs of chronic liver failure was confirmed upon physical examination. Routine laboratory tests for primary liver disease were all normal except for increased ALAT levels (86 U/l while treated with a statin). Hypercholesterolaemia (total cholesterol 9.0 mmol/l, total cholesterol/HDL ratio 16.3) was also present. Stool weights were normal with normal fat content. Varices at the rectosigmoid junction were found upon colonoscopy, and gastroscopy performed at a later stage confirmed the diagnosis of portal hypertension (grade I). Liver biopsy (*figure 1A-C*) showed periportal and bridging fibrosis, without signs of cirrhosis. The hepatocytes were enlarged and contained non-uniform microvesiculae. Foamy macrophages were seen both in the portal tracts and in the parenchyma. Bone marrow cytology showed few, but clearly distinguishable, 'sea-blue histiocytes' and vacuolated macrophages, indicators of, among others, a lysosomal storage disease (*figure 1D*).[5-7] In line, chitotriosidase levels were also increased (325 nmol/ml/h; reference < 200 nmol/ml/h), indicating macrophage accumulation.[5,8] Microscopy findings were consistent with the diagnosis of Niemann-Pick disease (type B or C) but the finding of normal sphyngomyelinase activity in leucocytes and the negative filipin staining of cultured fibroblasts refuted these diagnoses. These results, combined with reduced cholesterol esterification, did not, however, exclude CESD. Therefore, LAL activity

Figure 1.
A. *Liver needle biopsy. Portal tract. White arrows indicate an enlarged portal tract containing macrophages. Black arrows indicate hepatocytes in the surrounding parenchyma with non-uniform microvesicular fat droplets. (Periodic acid Schiff after diastase digestion, 20X)*
B. *CD68 staining highlights the CD68 positive macrophages in a portal tract (brown colour, immunostaining with anti CD68 antibody 40X)*
C. *Liver needle biopsy showing an enlarged portal tract with bridging fibrosis (indicated by arrow) (Masson Trichrome 10x)*
D. *Bone marrow cytology of the CESD index patient. Arrow indicates a sea-blue histiocyte (Wright's stain 400x)*



was subsequently measured in fibroblast cultures and was found to be strongly decreased (0.2 nmol/h per mg fibroblast protein in the proband versus 8-15 nmol/h per mg fibroblast protein in control cultures). Since mutations in *LIPA* are known to cause reduced LAL activity and, as a consequence CESD, we used targeted DNA diagnostics (RT-PCR) of the *LIPA* gene to confirm the diagnosis. This revealed compound heterozygosity for the E8SJ mutation and the T1107G mutation in exon 10 in *LIPA*.

Two brothers were also diagnosed with CESD (*figure 2*). Neither of them had sought medical attention for CESD-related symptoms and might have remained undiagnosed if the proband had not been identified as a CESD patient. The portal hypertension in the proband was successfully treated with propanolol. The diarrhoea ceased after treatment with fibres (psyllium). After confirmation of the diagnosis of CESD, treatment was started with a statin. Serum cholesterol and total cholesterol/HDL ratios decreased twofold.

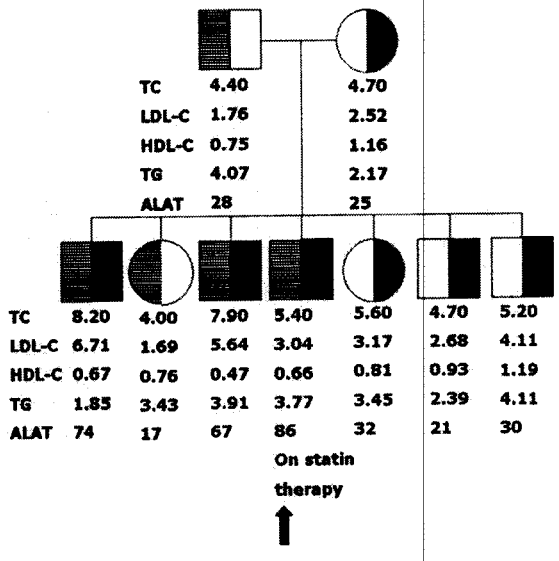
DISCUSSION

We here describe two family cases of CESD. Case 1 presented with a typical phenotype of familial hypercholesterolaemia. In case 2, the combination of bone marrow cytology ('sea-blue histiocytes' and vacuolated macrophages), liver histology (microvesicular steatosis, bridging fibrosis and lipid laden macrophages), and a combined hyperlipidaemia finally led to the diagnosis of CESD. These cases illustrate the variability of phenotypes of patients with CESD and emphasise that in patients with hypercholesterolaemia of unknown origin or unexplained hepatosplenomegaly, *LIPA* mutations could be present. Moreover, our two cases clearly show the differential diagnostic pathways that can ultimately lead to the identification of a uniform molecular pathological substrate of a disease. It is of note that heterozygosity for *LIPA* mutations is not associated with clinical signs and symptoms. The E8SJ mutation is the most frequently found mutation in CESD patients, leading to skipping of exon 8 and resulting in only a fraction of the normal hLAL enzyme levels.[9,10] The T1107G mutation in exon 10 of *LIPA* causes a premature stop codon that consequently results in the synthesis of a truncated LAL protein. Together with the exon 8 mutation this results in a nearly complete abolishment of hLAL activity in the compound heterozygotes. To the best of our knowledge, this mutation has not been previously described.[2]

Since elevated LDL-C and triglyceride levels as well as decreased HDL-C levels are associated with development of cardiovascular disease (CVD), CESD patients are likely to be at increased CVD risk.[11] The low number of patients known thus far, however, preclude us from drawing firm conclusions in this regard. Although no specific therapy for CESD is available to date, current treatment of CESD patients comprises treatment of dyslipidaemia with statins, given the established beneficial effect on both lipids and CVD risk.[11] The effect of statin therapy on the extent of hepatic cholesteryl-ester accumulation is, however, unknown.[10]

Sebelipase alpha (SBC-102; Synageva BioPharma Corporation, Lexington, Massachusetts, USA) is a recombinant human LAL enzyme that is currently in the clinical stage of development and that has been tested in nine CESD patients to date.[12,13] In this population, sebelipase alpha has shown to significantly reduce ALAT and aspartate aminotransferase (ASAT) levels by 52% (44 U/l) and 36% (20 U/l), respectively after 12 weeks (4 weekly infusions followed by 4 bi-weekly infusions of 0.35 mg/kg – 3 mg/kg).[13] These decreases were shown to sustain for up to 52 weeks of treatment in seven patients, where ALAT levels were decreased by 58% (49 U/l) and ASAT levels were decreased by 40% (23 U/l) ($p = 0.016$) from baseline (4 weekly infusions followed by 24 bi-weekly infusions). Besides the reductions in transaminase levels, sebelipase alpha has also shown to significantly reduce lipid and lipoprotein levels. After 52 weeks of treatment, total cholesterol levels decreased by 1.84 ± 0.80 mmol/l ($p = 0.016$), LDL-C decreased by 1.89 ± 0.80 mmol/l ($p = 0.016$) and triglyceride levels decreased by 0.81 ± 0.41

Figure 2. *Pedigree of family 2*



Stripes indicate T1107G mutation in exon 10 in *LIPA*. Black indicates G934A (E8SJ) mutation in exon 8 in *LIPA*. White indicates wild-type *LIPA* allele. Lipoprotein levels are in mmol/l; ALAT levels in U/l. ALAT = alanine aminotransferase; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol; TC = total cholesterol; TG = triglycerides.

mmol/l (p = 0.047). Infusion reactions were only observed in one patient, who was finally successfully re-challenged.[13] Sebelipase alpha is currently being tested in a placebo-controlled phase III trial (ARISE; NCT 01757184).

The non-specific phenotypic characteristics of CESD and the (partial) overlap of phenotypic features with other cardiovascular, liver and metabolic diseases illustrate the difficulty in diagnosing patients with CESD and suggest that patients with this disease might be missed or remain undiagnosed. This is supported by the large discrepancy between the number of reported CESD cases in the literature and the estimated prevalence of this disease.[3,10] The large variability of CESD phenotypes, as illustrated in this case report, shows that more insight into the natural clinical course of homozygosity/compound heterozygosity for *LIPA* is warranted, since this will influence the identification of patients who will have benefit from future enzyme replacement therapy.

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