Pelger-Huët anomaly and Greenberg skeletal dysplasia: LBR-associated diseases of cholesterol metabolism

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ABSTRACT
Lamin B Receptor (LBR) is an inner nuclear membrane protein associated with the rare human diseases Pelger-Huët anomaly and Greenberg skeletal dysplasia. A new study has used CRISPR/Cas9-mediated genetic manipulations in a human cell system to determine that the molecular etiology of these previously poorly understood disorders is a defect in cholesterol synthesis due to loss of LBR-associated sterol C14 reductase activity. The study furthermore determined that disease-associated LBR point mutations reduce sterol C14 reductase activity by decreasing the affinity of LBR for the reducing agent NADPH. Moreover, two disease-associated LBR truncation mutants were found to be highly unstable at the protein level and are rapidly turned over by a novel nuclear membrane-based protein quality control pathway. Thus, truncated LBR variants can now be used as model substrates for further investigations of nuclear protein quality control to uncover possible implications for other disease-associated nuclear envelopathies.

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Introduction
Lamin B Receptor (LBR) is a bi-functional polytopic membrane protein of the inner nuclear membrane (INM).1,2 The N-terminus of LBR resides inside the nucleus and directly interacts with both chromatin and the nuclear lamina, the meshwork of intermediate filaments that underlies and supports the inner nuclear membrane (Fig. 1).2-5 This is the function for which the protein is named, but LBR has a second, seemingly unrelated role in sterol metabolism as the C-terminal, multi-pass transmembrane domain of the protein is a functional C14 sterol reductase.6,7 This second function of LBR has until now been somewhat puzzling as human cells contain another C14 sterol reductase called TM7SF2 or DHCR14 which, like most other enzymes in the cellular sterol biogenesis pathway, resides in the ER membranes and is responsive to the sterol regulatory element-binding protein (SREBP) pathway.8-10 LBR, conversely, is constitutively expressed, unresponsive to the SREBP pathway, and resides instead at the INM.8,11 Thus it has long been assumed that TM7SF2/DHCR14 is the primary cellular C14 sterol reductase while LBR provides at most redundant or cell type/developmental stage specific sterol biogenesis activity. However, a recent study by Tsai et al. has demonstrated not only that the sterol reductase activity of LBR is strictly required for viability under sterol-restrictive growth conditions in several human cell types, but also that two rare human diseases associated with mutations in LBR, Pelger-Huët anomaly and Greenberg skeletal dysplasia, are caused by the loss of LBR-based sterol reductase activity.11

Lamin B receptor and human disease
Mutations to proteins of the nuclear lamina are known to cause a wide spectrum of human genetic diseases with phenotypes including muscular dystrophy, lipodystrophy, cardiomyopathy and the premature aging disorder Hutchinson-Gilford progeria syndrome.12-15 Collectively, these disorders are known as nuclear laminopathies, but in most cases the molecular pathways by which particular laminopathic mutations manifest as specific disease phenotypes...
remain to be determined. Two known human diseases are associated with mutations in LBR: Pelger-Huët anomaly and Greenberg skeletal dysplasia (Table 1). Pelger-Huët anomaly is a relatively benign autosomal dominant disorder characterized by hypolobulation of the normally hyperlobulated nuclei of granulocytes, a type of white blood cell. Although studies of cells derived from the partial LBR knockout ic/ic mouse model have demonstrated a significant defect in the growth and derivation of myeloid progenitors, the multipotent cells from which all bone marrow-based blood cells are derived, individuals displaying Pelger-Huët anomaly in granulocytes are otherwise asymptomatic. Conversely, Greenberg skeletal dysplasia (also known as hydrops-ectopic calcification-moth-eaten or HEM skeletal dysplasia) is a perinatally lethal autosomal recessive condition characterized by highly abnormal bone development (with abnormal calcification and a “moth eaten” appearance) and excessive fluid accumulation (fetal hydrops). Interestingly, some LBR mutations found in the heterozygous state in individuals displaying Pelger-Huët anomaly have also been found in HEM/Greenberg dysplasia fetuses, and Pelger-Huët anomaly has been observed in Greenberg skeletal dysplasia parents, suggesting that at least in some cases, the two disorders represent different allelic states of the same genetic lesion, rather than distinct and unrelated genetic diseases. As LBR is a bi-functional protein, with both nuclear lamina/chromatin binding and C14 sterol reductase activities, there has been some debate as to whether these disorders should be classified as nuclear laminopathies or as diseases of cholesterol metabolism. Notably, elevated levels of the direct upstream cholesterol intermediate acted upon by the C14 sterol reductases LBR and TM7SF2 were detected in a Greenberg dysplasia fetus, raising the question of why a deficiency in LBR is not

![Figure 1. Localization and topology of LBR and Emerin at the inner nuclear membrane. Disease-associated LBR point mutations N547D and R583Q are indicated by filled circles and frameshift mutations by a Δ symbol at the point of truncation. (INM) inner nuclear membrane, (ONM) outer nuclear membrane, (PNS) perinuclear space.](image)

### Table 1. Congenital diseases associated with Lamin B receptor.

| LBR Mutation (gene) | LBR Mutation (protein) | Type | Phenotype (heterozygous) | Phenotype (homozygous) | Reference |
|---------------------|------------------------|------|--------------------------|------------------------|-----------|
| c.1639A>G          | p.N547D                | point mutation | Unknown                  | Greenberg Dysplasia    | Clayton et al 26 Konstantinidou et al 18 Tsai et al 11 |
| c.1748G>A          | p.R583Q                | point mutation | No Phenotype             | Greenberg Dysplasia    | Clayton et al 26 Tsai et al 11 |
| c.1402delT         | p.Y468fsX475           | frameshift    | Unknown                  | Greenberg Dysplasia    | Clayton et al 26 Tsai et al 11 |
| c.1599-1605 TCTTC TA<CTAGAAG | p.X534 | nonsense      | Pelger-Huët Anomaly      | Greenberg Dysplasia    | Waterham et al 22 Tsai et al 11 |
| c.32delTGT         | p.V11fsX24             | frameshift    | Pelger-Huët Anomaly      | Greenberg Dysplasia    | Clayton et al 26 |
| c.355C>T           | p.P119L                | point mutation | Pelger-Huët Anomaly      | Unknown                | Best et al 19 |
| c.1706G>G          | p.P569R                | point mutation | Pelger-Huët Anomaly      | Unknown                | Best et al 19 |
| c.1308G>A          | p.X436                 | nonsense      | Pelger-Huët Anomaly      | Unknown                | Hoffman et al 16 |
| c.1173del          | p.G382fsX393           | frameshift    | Pelger-Huët Anomaly      | Unknown                | Hoffman et al 16 |
| c.1129C>T          | p.X377                 | nonsense      | Pelger-Huët Anomaly      | Unknown                | Hoffman et al 16 |
| c.500G>C-501-504 delCCTT | p.S167TfsX176 | frameshift    | Pelger-Huët Anomaly      | Unknown                | Hoffman et al 16 |
| IVS11-9A>G         | splice acceptor        | Pelger-Huët Anomaly | Unknown                | Best et al 19 |
| IVS2-2A>G          | splice acceptor        | Pelger-Huët Anomaly | Unknown                | Hoffman et al 19 |
| IVS12-5-10del      | splice acceptor        | Pelger-Huët Anomaly | Unknown                | Hoffman et al 19 |
| IVS11+1G>A         | splice donor           | Pelger-Huët Anomaly | Unknown                | Hoffman et al 19 |
| IVS13-2A>G         | splice acceptor        | Pelger-Huët Anomaly | Unknown                | Hoffman et al 19 |
compensated for by the putative primary C14 sterol reductase TM7SF2, in which no defect was detected. Thus the molecular etiology of these related disorders has until now remained unclear.

**Pelger-Huët anomaly and Greenberg skeletal dysplasia are loss-of-function diseases of cholesterol metabolism**

Using a combination of CRISPR/Cas9 mediated knockout and Flp/Frt recombination-based gene integration, we have established a system for conducting classical genetics in a human cell system and revealed the molecular basis of the rare human diseases Pelger-Huët anomaly and Greenberg skeletal dysplasia. We determined that LBR is required for cell viability under sterol-restrictive growth conditions in 3 different human cell lines and directly detected a decrease in cellular cholesterol content in LBR knockout cells, challenging the assumption that the C14 sterol reductase TM7SF2/DHCR14 is a redundant enzymatic activity. Interestingly, we also found no defect or change in the structure of the nuclear lamina in an LBR knockout cell line, arguing against a primarily structural role of LBR at the INM, albeit only in a tissue culture cell system.

Furthermore, we employed Flp/Frt based recombination to re-introduce both wild-type and disease-associated LBR alleles into an LBR knockout cell background, achieving moderate protein expression under doxycycline control and allowing for a careful analysis of both genotype and phenotype. Using this system, we determined that re-integration of wild-type LBR fully rescues the observed cholesterol auxotrophy phenotype, but that 4 separate LBR alleles associated with Pelger-Huët anomaly and Greenberg skeletal dysplasia (Table 1)(Fig. 1) do not.

Further investigation of these four disease-associated LBR alleles revealed two separate and distinct mechanisms by which LBR mutation leads to a defect in cholesterol production. By comparison to a published structure of a related bacterial sterol C14 reductase, we found that the LBR point mutations LBR N547D and LBR R583Q map to the NADPH binding pocket. As NADPH is the essential cofactor used by C14 sterol reductases to convert a carbon-carbon double bond in the cholesterol intermediate 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol to a single bond, any mutation which decreases the binding affinity of the enzyme for NADPH is expected to significantly decrease its reductive capacity. Indeed, we found that LBR R583Q has an approximately 7-fold decrease in
affinity for NADPH compared to wild-type LBR, while LBR N547D showed an almost 12-fold decrease in NADPH binding capacity, resulting in a near-complete loss of de novo cholesterol synthesis.\textsuperscript{11}

Conversely, the two LBR truncation mutants, both of which result in the omission of several transmembrane helices from the C-terminus of the protein (Fig. 1), are highly unstable and are rapidly turned over by cellular protein quality control machinery, resulting in an extremely low steady-state protein expression level. Remarkably, it appears that truncated LBR is degraded by a mechanism that resembles the traditional ER-associated protein degradation (ERAD) pathway in that LBR ubiquitylation, a functional proteasome, and a functional p97 motor are required\textsuperscript{30,31} (Fig. 2). However, unlike in the canonical ERAD pathway, which has until now only been found in the ER membranes, truncated LBR is first trafficked to the nuclear membranes before being dislocated directly into the nucleoplasm, where it is presumably degraded by nuclear proteasomes. This is, to our knowledge, the first instance in which an INM-based ERAD-like pathway has been observed in higher eukaryotes, and represents a second molecular mechanism by which LBR-associated disease mutations result in a loss of sterol C14 reductase functionality.

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No potential conflicts of interest were disclosed.

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