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Liddle’s Syndrome: Literature Review of Genetics, Pathophysiology and the Future of Research and Potential Cure

Daniel McKenzie
Introduction

Liddle’s Syndrome is a genetically inherited form of hypertension. It is a rare condition with few diagnoses throughout the world each year, partially due to its common misdiagnosis as aldosteronism. Patients with Liddle’s Syndrome often experience hypertension, or high blood pressure, and in many cases hypokalemia, or low potassium levels in the blood. Hypertension is a common condition that increases the risk of heart attack, stroke, aneurysms, heart failure, kidney disorders, and many other ailments. Hypokalemia is a serious problem that can cause many neuromuscular symptoms including muscle weakness and cardiac arrhythmias. Liddle’s Syndrome is caused by a genetic mutation in the subunit of the epithelial sodium channel (ENaC) found in the distal nephron of the kidney. The ENaC has three subunits (α, β, γ) and mutations are typically found in the β, and γ subunits of the channel. This genetic mutation causes the ENaC to be retained in the membrane when it would normally be degraded, resulting in over activity of the channel. This leads to the main cause of the hypertension, excess sodium reabsorption. The excess reabsorption of the sodium leads to water retention and an increase in the volume of blood in the body. This expansion in volume is believed to be the cause of the hypertension. The disease is inherited through an autosomal dominant pattern, meaning that the offspring of affected individuals have a 50% chance of also being affected. The treatment of the disease is commonly a sodium-restricted diet as well as the use of amiloride or triamterene diuretics. In rare cases, kidney transplantation is used to treat the disease. Throughout this paper we will go into more depth about each part of the disease and how they all are connected. Eventually leading to how the use of gene/genetic therapy advances could help shape the future of the diagnosis, treatment, and potentially cure of Liddle’s Syndrome. We will look into other similar diseases and how the use of gene therapy is used in them and how similarities in the
diseases could support that the therapy would be beneficial to those affected by Liddle’s Syndrome.

**Genetics of Liddle’s Syndrome**

Liddle’s Syndrome is an inherited form of hypertension. This inheritance follows an autosomal dominant pattern. An autosomal dominant pattern of inheritance means that if the offspring receives the abnormal gene from only one parent, they will exhibit the traits of the disease. This means that if a single parent is affected by the mutation, the offspring has a 50% chance of acquiring the mutation and ultimately the disease. In the case of Liddle’s Syndrome, the mutation that is inherited by offspring affects Epithelial Sodium Channel (ENaC) subunits. These subunits are encoded by *SCNN1A, SCNN1B,* and *SCNN1G* genes. The last letter in these names corresponds to the subunit they encode, α β and γ (K.-Q. Yang et al., 2014). Mutations in *SCNN1B* and *SCNN1G* and therefore the β and γ subunits have been found to be linked to Liddle’s Syndrome; work is ongoing to determine if mutation of the α subunit plays a role in the development of Liddle’s Syndrome. The *SCNN1B* gene was found to have a premature stop codon that truncates the C-terminus of the β subunit, and this was the first report of Liddle’s Syndrome being caused by a gene mutation (Shimkets et al., 1994). The *SCNN1G* gene was found to have a mutation that truncated the C-terminus of the gene of the γ subunit (Hansson et al., 1995). The reported cases of Liddle’s Syndrome all exhibit missense, nonsense, or frameshift mutations (Gao et al, 2013). This premature truncation of the protein can cause alterations in the amino acid sequence within the PY motifs of the subunits. The PY motifs are found in the extracellular loop of the α, β, and γ subunits of the ENaC. These domains are important because they are the binding sites for the WW domains of NEDD4-2, a ubiquitin ligase protein. When the WW domains bind to the PY motifs, NEDD4-2 binds to the subunits and initiates the ubiquitylation
process. The ubiquitylation process involves the endocytosis, internalization and removal of ENaCs from the membrane. Under normal conditions, the NEDD4-2 protein degrades the ENaCs so that the Na\(^+\) levels remain in the homeostatic range. In the case of Liddle’s Syndrome, the ENaC is not removed. The ENaC is not removed because the PY motifs are altered and the NEDD4-2 cannot bind. When the NEDD4-2 ubiquitin ligase cannot bind, the subunit is not signaled for endocytosis and degradation (Bhalla and Hallows, 2008). This causes an excess amount of ENaCs to be found on the membrane. When ENaC is overexpressed, Na\(^+\) reabsorption is elevated in the distal tubule and this leads to issues in the regulation of Na\(^+\) as well as plasma volume. The increase in the Na\(^+\) and plasma volume ultimately leads to the hypertension experienced by those with Liddle’s Syndrome.

**Epithelial Sodium Channels (ENaC)**

Epithelial sodium channels (ENaC) in the distal nephron are ion channels located in the apical or luminal membrane, the epithelial cell plasma membrane that faces the lumen of the distal nephron. These channels are also present in the epithelial cells of tissues including the lungs, exocrine glands and colon. In the lungs, epithelial sodium channels play a role in lung fluid clearance. In the exocrine glands, epithelial sodium channels play a role in the control of blood pressure through regulation of sodium balance and blood volume. In the colon, epithelial sodium channels play a role in transepithelial sodium reabsorption by aldosterone and protect against salt overload (Rossier, 2014). In the distal nephron, the channels facilitate Na\(^+\) reabsorption. The channel is composed of three subunits α, β, and γ. These three subunits are similar in structure and contain two transmembrane domains (TM1 and TM2), a large extracellular loop, short C-terminal domain and N-terminal domain. The large extracellular loop is where the proteolytic sites are located. These proteolytic sites are where the ubiquitylation process takes place and this
causes the channel to undergo endocytosis and degradation resulting in the removal of the channel from the membrane. The C-terminal domain of the subunits contain an internalization motif (Yang, Xiao, Tian, Gao, & Zhou, 2014). When an epithelial sodium channel is functioning normally, it allows for the movement of sodium (Na⁺) down its concentration gradient into the cell. In normal conditions, the concentration of Na⁺ outside the cell is much larger than inside the cell allowing for the passive movement through the ion channel. This larger extracellular concentration is maintained through the activity of the Sodium/Potassium ATPase (Na⁺/K⁺ ATPase). The Na⁺/K⁺ ATPase uses energy in the form of ATP (adenosine triphosphate) to power the movement of three molecules of sodium out of the cell and movement of two potassium molecules into the cell, both against their concentration gradients. In normal cell function, these channels are degraded to limit too much sodium from entering the cell.

In the case of Liddle’s Syndrome, these ENaC’s contain a gain-of-function mutation that causes them to not be degraded and allows for continued entry of sodium into the cell. This creates an imbalance in Na⁺ homeostasis. The elevated Na⁺ reabsorption can cause changes in the blood volume and pressure, which are also partially regulated by aldosterone and hormones (Rossier, 2014). The high blood pressure exhibited in patients with Liddle’s Syndrome can be partially attributed to this gain-of-function mutation in the epithelial sodium channels.

Nephron
The kidney is composed of three parts: pelvis, medulla, and cortex. The pelvis is the innermost portion of the kidney and it is responsible for the collection of filtrates from the calyces and funneling these into the ureter to be excreted as urine. The dark outer cortex and paler inner medulla of the kidney contain some vasculature but are primarily composed of large numbers of epithelial-lined tubules called nephrons. The nephron is the functional unit of the kidney and is
The nephron is composed of the glomerulus, proximal tubule, Loop of Henle, distal tubule, and collecting ducts. The glomerulus is the first part of the nephron. The glomerulus is the bundle of capillaries in the renal corpuscle that play a role in filtration of the blood. The next component of the nephron is the proximal tubule. The proximal tubule is the primary location for reabsorption. In the proximal tubule, water, Na\(^+\), solutes, glucose, amino acids, and bicarbonate are reabsorbed. The proximal tubule also has a large number of mitochondria to make the adenosine triphosphate (ATP) necessary for the Na\(^+\)/K\(^+\) ATPase. The Na\(^+\)/K\(^+\) ATPase is an active transporter that uses ATP to move three Na\(^+\) out of the cell, and two K\(^+\) into the cell, both against their concentration gradients. The next component of the nephron is the Loop of Henle. The Loop of Henle is composed of a descending and ascending limb. The descending limb of the Loop of Henle reabsorbs water and this is done passively meaning that the water is moving down its concentration gradient and is not using energy. The descending limb is impermeable to sodium. The ascending limb is the location of Na\(^+\) reabsorption, and it is impermeable to water. The Na\(^+\) also moves passively down its concentration gradient without the use of energy. The next part of the nephron is the distal tubule. The distal tubule is the site of the fine tuning of the reabsorption. Na\(^+\) and water are the main components that can be reabsorbed in the distal tubule. The last component is the collecting duct. The collecting duct is the final site of reabsorption as it is the final location to fine tune the contents. The contents are ultimately excreted via urine.
ENaCs are located in the distal nephron, collecting tubule, and collecting duct and they play a role in the fine tuning of the Na\(^+\) reabsorption. The ENaC channels are specifically located in the apical, or luminal membrane of the epithelium facing the tubular fluid. In the case of Liddle’s Syndrome, the ENaCs are over-expressed and excessive Na\(^+\) is reabsorbed (Verouti et al., 2015). Through mechanisms discussed later, this can also result in increased K\(^+\) secretion into the urine, which depletes extracellular K\(^+\) concentration and can cause hypokalemia.

Ubiquitylation of Epithelial Sodium Channels (ENaC)

As mentioned in previous sections ENaCs play an important role in the regulation of Na\(^+\) in the cells of the kidney and other epithelia. Ubiquitylation involves the endocytosis, sorting and recycling of ENaCs and therefore is essential to the regulation of Na\(^+\) reabsorption indirectly.
Ubiquitylation is a process that includes three separate enzymes E1, E2, and E3. Ubiquitin activating enzyme (E1) is responsible for the activation of ubiquitin via the creation of a thioester bond using ATP (Haas et al., 1983). Following the activation of the ubiquitin molecule, it is then transferred to the ubiquitin conjugating enzyme (E2) via an active cysteine (Jentsch, 1992; Hershko and Ciechanover, 1998). Following this second step, the ubiquitin molecule is finally added to the substrate via a ubiquitin protein ligase (E3) (Hershko and Ciechanover, 1998). Ubiquitin protein ligase (E3) allows for substrate specificity and the ability to recognize certain substrates through different protein-protein interactions. The E3 found in the ubiquitylation process for ENaCs is part of the E3 family called HECT (homologous to the E6-AP C terminus) and the specific member of this family is called NEDD4-2. The NEDD4-2 protein consists of four WW domains, a C2 domain, and a HECT domain as shown below.

![Structural Arrangement of NEDD4-2 Protein](image)

The WW domains are 35–40 amino acid sequences that have two conserved tryptophan residues (Andre and Springael, 1994; Hofmann and Bucher, 1995). The WW domains generally bind to PY motifs of the sequences of the subunit and the multiple domains suggest that they can interact with more than one protein at a time. The C2 domain plays a role in binding to the lipid membranes (Scheffner and Kumar, 2014). The HECT domain as discussed earlier is a ubiquitin protein ligase that places the ubiquitin molecule on the sequence to signal the degradation of the molecule. Ubiquitylation is an important process because it down-regulates the action of ENaC by removing the channels from the membrane. In order to remove the ENaCs from the channel, the WW domains bind directly to the PY motifs found in the large extracellular loop of the
ENaC subunits, specifically the $\beta$ and $\gamma$ subunits (Snyder et al., 2004). This allows for the ubiquitin to be bound to the sequence and allow for it to undergo the process of endocytosis, sorting, and recycling. This removes the ability to take $\text{Na}^+$ into the cell for that channel.

In the case of Liddle’s Syndrome, the sequences in the extracellular loop of the $\beta$ and $\gamma$ subunits are mutated (Bhalla and Hallows, 2008). Due to this mutation, the NEDD4-2 complex, specifically the WW domains, cannot bind to the ENaC subunit sequence and the ENaCs are not degraded. Since these ENaCs are not degraded more $\text{Na}^+$ is allowed to enter the cell via these channels resulting in overexpression of the ENaC. The image below shows the inactivation of NEDD4-2 complex and how it should bind and induce degradation of the ENaC.

![Diagram of ENaC regulation by NEDD4-2](image)

Figure C. NEDD4-2 regulation of ENaC (Goel et al., 2015). In a high cytosolic Na$^+$ environment, Na$^+$ uptake is not required. When Na$^+$ uptake is not required WW3 and WW4 interact with the subunits of the ENaC leading to the binding and ultimately degradation of the ENaC. In the case of Liddle’s Syndrome this mechanism is disabled and the ENaC are not degraded leading to increased ENaC on the surface and greater ENaC activity. When Na$^+$ uptake is active, NEDD4-2 is inactive. The inactivation is achieved through the actions of Sgk1 (serum glucocorticoid-inducible kinase) and Akt (PKB) phosphorylating NEDD4-2. The phosphorylation of NEDD4-2 by these kinases allows for the binding of 14-3-3, which inactivates NEDD4-2.
Hypokalemia

Liddle’s Syndrome is a disease typically associated with the increased reabsorption of Na\textsuperscript+ via a constitutively, or continuously, expressed ENaC found in the luminal membrane of the distal nephron. A consequence of this increased Na\textsuperscript+ reabsorption is that it can cause hypokalemia in patients. Hypokalemia is defined as low blood potassium (K\textsuperscript+) concentration. While hypokalemia is not a universal finding in all cases of Liddle’s Syndrome it is found in a number of cases. Hypokalemia experienced in Liddle’s Syndrome patients is due to elevated levels of K\textsuperscript+ excretion in urine (Botero-Velez et al., 1994). This elevated level of K\textsuperscript+ excretion could be attributed to the excess Na\textsuperscript+ reabsorption via luminal membrane ENaC and consequently increased activity of the basolateral Na\textsuperscript+/K\textsuperscript+ ATPase. The increased Na\textsuperscript+/K\textsuperscript+ ATPase activity will move K\textsuperscript+ from the extracellular space into epithelial cells lining the nephron and from there promote urinary K\textsuperscript+ secretion, resulting in hypokalemia. In addition to the activity of the Na\textsuperscript+/K\textsuperscript+ ATPase, the fulfillment of the Law of Electroneutrality results in excess K\textsuperscript+ excretion. When the ENaC moves more Na\textsuperscript+ into the cell, the positive charge within the cell increases. This increase in the positive charge must be restored to the homeostatic levels and in order to achieve this the K\textsuperscript+ ions move out of the cell through K\textsuperscript+ channels in the luminal membrane and into the tubular fluid, thereby increasing K\textsuperscript+ secretion.
The table shown above is from the index case of Liddle’s Syndrome. The proband was a 16 year old female, who exhibited both severe hypertension and hypokalemic metabolic alkalosis. The proband also had two siblings with similar abnormalities. The proband eventually underwent kidney transplantation after renal function deteriorated. In the table shown above, all of the symptoms typically associated with Liddle’s Syndrome are present in the proband. The blood pressure is elevated to 180/110 mmHg in the first measurement and following transplantation of the kidney it is lowered to 140/79 mmHg (Botero-Velez et al., 1994). This hypertension is normally associated to the persistent volume expansion (Liddle et al., 1963). The urinary sodium excretion in the 1962 measurements was lower than the 1991 measurement indicating that the kidney was reabsorbing more Na\(^+\) than it should before the transplant. The measurements also show the reason for the hypokalemia experienced by the patient. The urinary potassium excretion was elevated compared to the level following transplantation and the serum levels were lower in the 1962 measurements than in the 1991 measurements (Botero-Velez et al., 1994). I believe the

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<tr>
<td>Blood pressure (mm Hg)</td>
<td>180</td>
<td>140</td>
<td>140</td>
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<tr>
<td>Systolic</td>
<td>110</td>
<td>80</td>
<td>79</td>
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<td>Diastolic</td>
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<td>9</td>
<td>9</td>
<td>150</td>
</tr>
<tr>
<td>Dietary sodium (mmol/day)</td>
<td>50</td>
<td>50</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Dietary potassium (mmol/day)</td>
<td>120</td>
<td>60</td>
<td>145±12</td>
<td>18±9</td>
</tr>
<tr>
<td>Urinary sodium excretion</td>
<td>80</td>
<td>30</td>
<td>47±13</td>
<td>39±2</td>
</tr>
<tr>
<td>Urinary potassium excretion</td>
<td>2.8</td>
<td>3</td>
<td>4.3</td>
<td>2</td>
</tr>
<tr>
<td>Trianistrene (mg per 8 hr)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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*Measurements from 1962 are those originally reported by Liddle et al., who did not report the systolic blood pressure during salt restriction. Values from 1991 were measured during inpatient studies performed 20 months after successful renal transplantation. P<sub>iso</sub> values are mean±SD for the first three days of each period of controlled dietary sodium intake.

Table 1. (Botero-Velez et al., 1994) Measurements of Blood Pressure and Electrolyte excretion in the proband patient. The table is showing the proband before kidney transplantation in 1962 and following kidney transplantation in 1991 and 1992. This is showing that before the kidney transplant the patient experienced increased blood pressure, a decrease in sodium excretion in 1962 when compared with 1991, and an elevated level of potassium excretion in 1962. These are all important indicators of Liddle’s Syndrome. The improvement following transplantation show that the Liddle’s Syndrome symptoms could be attributed to issues related to the kidneys and its components.
measurements from this proband patient indicate that the hypokalemia experienced in patients can be attributed to the disturbed of Na\(^+\) and K\(^+\) transport in the distal tubule. The maintenance of Na\(^+\) and K\(^+\) concentrations is normally attributed to the Na\(^+\)/K\(^+\) ATPase. In this effort to maintain the gradients the Na\(^+\)/K\(^+\) ATPase could be escalating the problem due to the excess Na\(^+\) in the cytoplasm due to the mutated ENaCs. In order to help treat the hypertension and hypokalemia associated with Liddle’s Syndrome, the drugs amiloride and triamterene have been administered and been effective (Liddle et al., 1963). These drugs are diuretics that act on the collecting tubules. They act by directly blocking the Na\(^+\) channels to prevent reabsorption and they also reduce K\(^+\) excretion. Spironolactone is another drug that was attempted as a treatment, but it was found to be ineffective. This caused the argument that the syndrome was caused by an unidentified mineralocorticoid to lose support (Botero-Velez et al., 1994).

**Hypertension**

Hypertension, also known as high blood pressure, is a common condition that affects many people around the world. It is commonly caused by risk factors like age, race, obesity, stress, tobacco usage, excessive alcohol use, and other conditions. Many of these factors can be managed by lifestyle changes and this can help prevent hypertension. Hypertension is an issue for the health of individuals because increased volume of blood or pressure increases the stress on the heart and blood vessels. The increased stress due to hypertension on the blood vessels over a long period weakens the vessels and increases the risk for medical conditions. The risk for heart attack, stroke, aneurysms, heart failure, kidney function disorders, and other complications are increased in patients with hypertension. Hypertension due to Liddle’s Syndrome is atypical in that the hypertension is not caused by the lifestyle choices like most forms of hypertension. In Liddle’s Syndrome, hypertension is believed to be caused by a persistent volume expansion of
the blood in the patients (Liddle et al., 1963). The increase in the volume of blood is due to the increased Na$^+$ reabsorption due to the constitutive expression of ENaCs (Rossier, 2014). The constitutive ENaCs reabsorb Na$^+$, which creates an osmotic gradient that reabsorbs water too. This water reabsorption increased the volume of the blood and therefore also the blood pressure. The ENaCs are constitutively expressed in patients with Liddle’s Syndrome because of a mutation in the sequence of the subunits of the channel (Bhalla and Hallows, 2008). The mutation changes the sequence that is responsible for the signaling for degradation. This failure of the signaling causes the channel to remain in the membrane and allow for more Na$^+$ to be reabsorbed when it typically would not (Snyder et al., 2004). This is a gain-of-function mutation.

Summary of Liddle’s Syndrome Pathophysiology

Liddle’s Syndrome is a genetically inherited form of hypertension. The mutation is inherited via an autosomal dominant pattern, meaning that the offspring of those affected have a 50% chance of being affected. The mutation is located on the SCNN1A, SCNN1B, and SCNN1G genes (K.-Q. Yang et al., 2014). These genes code for subunits that comprise the epithelial sodium channel (ENaC). The mutations in the SCNN1B and SCNN1G genes cause change in the sequence that codes for the extracellular domain loops of the β and γ subunits of the ENaC. These mutations cause the β and γ subunits to be truncated (Bhalla and Hallows, 2008). The truncation causes the subunits to no longer have the PY motifs that are important for the binding of the ubiquitin protein ligase, NEDD4-2 (Snyder et al., 2004). When the NEDD4-2 ubiquitin protein ligase binds normally, the ubiquitylation process results in the endocytosis, internalization and removal of the ENaC. When the ENaC is not degraded, as in the case of Liddle’s Syndrome, it remains active in the membrane. The excess ENaC channels in the membrane cause a gain-of-function phenotype resulting in increased ENaC activity. The channels allow for excessive Na$^+$
reabsorption in the distal tubule of the nephron. The Na⁺/K⁺ ATPase normally controls the concentration gradients of the Na⁺ and K⁺ ions, but in the case of Liddle’s Syndrome the gradients are disturbed due to the increased number of ENaCs and increased Na⁺ transport. This excess Na⁺ reabsorption impacts the fluid regulation in the nephron, which is the functional unit of the kidney. Due to the excess Na⁺ reabsorption, increased water is reabsorbed, following the increased osmotic gradient, leading to an increase in plasma volume. The excess Na⁺ reabsorption also leads to hypokalemia, which is low blood potassium. The hypokalemia is caused by both the Na⁺/K⁺ ATPase pumping K⁺ out of the extracellular fluid and into the epithelial cells lining the distal nephron, and by the subsequent increase in secretion of that K⁺ into the tubular fluid following the requirement for the electroneutrality (the positive charge of the excess Na⁺ in the cell causes the cell to become more positive; in order to restore the charge of the cell to neutral the K⁺ is pumped out of the cell into the tubular fluid - this K⁺ is excreted in urine) leading to the low blood potassium levels (Botero-Velez et al., 1994). The excess Na⁺ reabsorption and hypokalemia are important factors that cause the hypertension that those affected with Liddle’s Syndrome experience. In order to help treat the hypertension and hypokalemia associated with Liddle’s Syndrome, the drugs amiloride and triamterene have been administered and been effective (Liddle et al., 1963). Changes in lifestyle to decrease the Na⁺ intake in the diet of the person are also important in the treatment. These seem to be effective treatments in most of the affected, but in extreme cases where the renal function cannot be controlled, it can cause deterioration of the kidney. The deterioration of the kidney can result in a need for kidney transplantation.

**Future Considerations: Gene/Genetic Therapy**
Liddle’s Syndrome is a disease caused by a genetic mutation and in many cases also presents hypokalemia. With advances in DNA sequencing methods and the emergence of techniques such as CRISPR that allow gene editing, genetic diseases like Liddle’s Syndrome may have a cure or at minimum be diagnosed earlier in life. The ENaCs that are affected by Liddle’s Syndrome are coded for by the \textit{SCNN1A, SCNN1B, and SCNN1G} genes (K.-Q. Yang et al., 2014). The \textit{SCNN1B} gene was found to have a premature stop codon that truncates the C-terminus of the \( \beta \) subunit, and this was the first report of Liddle’s Syndrome being caused by a gene mutation (Shimkets et al., 1994). The \textit{SCNN1G} gene was found to have a mutation that truncated the C-terminus of the gene of the \( \gamma \) subunit (Hansson et al., 1995). These mutations cause the lack of the PY domains that the NEDD4-2 ubiquitin ligase binds in order to remove the ENaCs (Bhalla and Hallows, 2008). Since we know that Liddle’s Syndrome is caused by these mutations of the \textit{SCNN1B} and \textit{SCNN1G} genes, it is possible to sequence the DNA of the offspring with parents that are affected by the disease. If we do this, we will be able to determine if the offspring are also affected earlier before the clinical symptoms present themselves. Proof of concept for this approach has been provided by a study of a family with an index case of Liddle’s Syndrome. 22 available at-risk subjects had genetic testing and 16 of them had endocrine testing. The DNA was extracted from whole blood leukocytes and was analyzed for the mutation that was found in the index case. In the study it was found that seven living relatives of the index case were found to have the mutation. Six of the mutation carriers had a history of hypertension and another two had hypertension during or just after pregnancy (Findling et al., 1997). This study shows that DNA sequencing can be a useful tool in diagnosing those that have a chance of inheriting Liddle’s Syndrome. The genetic mutation is able to be found in the sequence relatively easy, and those
that are found to have the mutation also show the symptoms that Liddle’s Syndrome is characteristic for.

Since we are able to determine the carriers of the disease via genetic testing, we know from which parent the Liddle’s Syndrome mutation is being passed. If the carrier is a male, there is potential for gene editing of spermatogonial stem cells (SSCs) that could fix the mutation and pass the corrected gene on to the offspring. In a study performed on mouse spermatogonial stem cells it was found that the CRISPR-Cas9 system could introduce genetic modifications into the SSCs. A few unique traits of SSCs are that they can self-renew and undergo spermatogenesis leading to production of numerous spermatozoa, and they can be maintained in vitro for long periods of time in medium supplemented with glial cell line-derived neurotrophic factor (Wu et al., 2015). Due to the ability to proliferate and be maintained in vitro, the researchers were able to screen the SSCs before insertion into the testes for unintended mutations. This limited the potential for unintended defects in the offspring, while still restoring the genetic mutation back to the normal sequence. This allowed for a 100% efficiency in producing healthy offspring. This technology shows promise in the genetic editing of mice and more models will need to be tested before humans, but this method is promising if the carrier of the disease is male. In the case of
Liddle’s Syndrome, this would provide the ability to restore the \textit{SCNN1B} and \textit{SCNN1G} genes to normal and not the truncated form that causes for the ENaCs to remain in the membrane.

Another method of gene/genetic therapy that has shown promise for the treatment of genetic diseases are transcription activator-like effector nucleases (TALENs). The TALENs comprise a nonspecific DNA-cleaving nuclease joined with a DNA-binding domain that allows for the targeting of almost any sequence (Joung and Sander, 2013). Theses TALENs induce double-strand breaks into specific DNA sites that are then repaired by mechanisms like non-homologous end joining (NHEJ) or homology-directed repair (HDR) that can be used to create sequence alterations and potentially fix the mutations causing genetic disease (Joung and Sander, 2013).

Liddle’s Syndrome is a genetic disease that results from missense, nonsense, or frameshift mutations (Gao et al., 2013). This variability of mutations that causes Liddle’s Syndrome can be addressed with TALENs because of the ability to change the DNA-binding domain of the nuclease to fit the specific mutation in the case. The main genetic cause of Liddle’s Syndrome is due to the premature truncation of the \textit{SCNN1B} and \textit{SCNN1G} genes causing the lack of the PY motifs that the NEDD4-2 ubiquitin ligase protein binds to degrade the ENaCs. If we could alter the sequence to remove the mutation and allow for the translation of the correct sequence to occur, we could potentially solve the problem of the ENaCs remaining in the membrane for too long. Recent studies using Zinc Finger Nucleases (ZFNs), which are an alternative to TALENs, have been used to correct genetic mutations responsible for sickle cell anemia and \(\alpha_1\)-antitrypsin disease in patient-specific induced pluripotent stem (iPS) cells that have been reprogrammed from fibroblasts. These studies show proof of principle that the correction of cells \textit{ex vivo} and reintroducing into patients could be a potential cure for genetic disease (Joung and Sander, 2013). The ability of TALEN’s to target essentially any DNA sequence should motivate
experimentation and refinement of this strategy that could eventually lead to a cure to genetic
diseases, like Liddle’s Syndrome.
References


