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Association of Epithelial Sodium Channel with Blood Pressure: A Systematic Review.

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ABSTRACT

To determine the role of epithelial sodium channel (ENaC) in increasing blood pressure. MEDLINE data base from January 1922 to July 2013 were systematically searched using a sensitive search strategy. Observational and clinical human studies were included in the review. Two reviewers independently evaluated studies for inclusion and extracted the data using a standardized data collection form. Eight studies were identified from the search. Certain population were involved, Chinese, Australian, Finland and certain parts of United State of America. Gene mutations in ENaC subunits are suggested to elevate blood pressure. Several molecular variants of ENaC subunits have been identified to suggest the association of ENaC with the prevalence of hypertension in certain population. However, these variants did not clearly demonstrate the link to the increased level of blood pressure. Few mechanistic pathways associated with ENaC expression need to be further elucidated through *in vivo* experiments.

Keywords: epithelium sodium channel, blood pressure, hypertension

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INTRODUCTION

Hypertension is a major, worldwide health problem with high prevalence and one of the major risk factor for stroke, myocardial infarction and end-stage renal disease. Until now, the genetic basis of hypertension is constantly explored in association with environment factors that may contribute to a higher level of blood pressure (Figure 1). This knowledge is crucial to develop a targeted blood pressure lowering agent which is specific to certain population of a specific region. By knowing the gene-environmental association, early screening for hypertension in high risk patients can be done and reduce the prevalence of the disease and its complication.

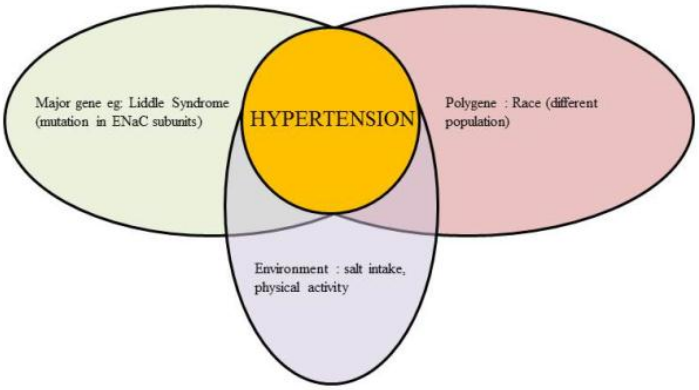

Figure 1: Factors contribute to hypertension

It is estimated as 30-50% of the variation in blood pressure might due to the genetic factors [1]. Thus, the development of hypertension is contributed by complex interplay of genetic alteration and environment factors in human population. Many genes have been associated with the elevated hypertension. One of the candidate genes is the amiloride-sensitive epithelial sodium channel (ENaC), which is responsible for controlling sodium reabsorption through the renal distal nephron. Imbalance of sodium reabsorption increases the blood volume and the blood pressure leading to Liddle's Syndrome [2].

ENaC is composed of three subunits: α-, β-, γ- which each have an intracellular carboxy (C) and amino (N) terminal and two membrane spanning domains connected by large extracellular loops [3,4,5]. ENaC can play a pivotal role to influence the risk for hypertension through Na^+ reabsorption in cortical collecting duct. The ENaC activity conveys to the adjustment of Na^+ balance and stimulation inversely proportional to the state of Na^+ balance when coupled with aldosterone [6].

Therefore, it is crucial to determine the association between ENaC and elevated blood pressure. We explored this association, from the bench (cell lines, animal models) to the bed (human) in this systematic review. We hope the information derived from this review will facilitate future studies to determine genetic predisposition of ENaC in different population leading to the development of hypertension and assist the improvement of pharmacogenomics in treating the disease.

METHODS/DESIGN

Search strategy

The following electronic databases were searched: MEDLINE (1922 to July week 2013). A sensitive search strategy with both control subheading and text terms relating to ENaC and elevated blood pressure was designed. Details of the search strategy are reported in Appendix 1.

Inclusion and exclusion criteria

All articles, published in English reporting the genes responsible for elevated blood pressure through ENaC, and mechanism of action of ENaC in human and animal studies were included. All types of hypertension

either primary or secondary were included. However, reviews, proceedings, letters and editorials are excluded from this study.

Data extraction

Two reviewers (NASI, NFAS) independently screened the title and abstract of all records identified by the search strategy. Full text copies of all potentially relevant articles were obtained and screened by the two authors. Any disagreements were resolved by discussion between the reviewers. A data extraction sheet was developed and refined accordingly. Outcome measured includes type of study, type of population, gene responsible for elevated blood pressure through ENaC and mechanism of action. For each study, two reviewers independently extracted data. Disagreement was resolved by discussion between the two reviewers. A table was developed to report the association between ENaC and elevated blood pressure.

Data synthesis

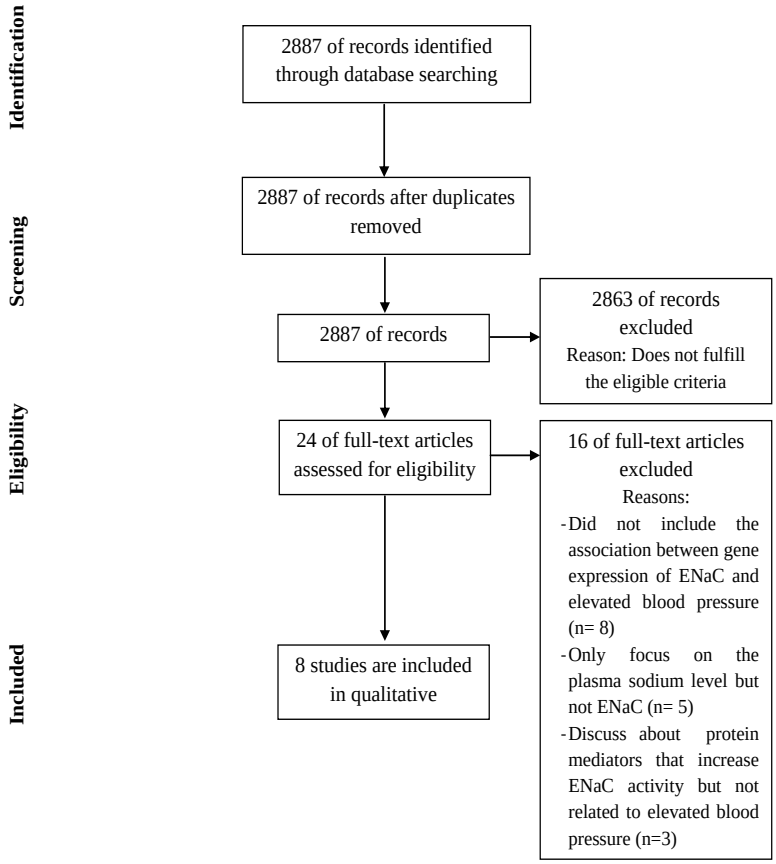

Figure 2: Flow chart of the selection process for the systematic review.

RESULTS

Study selection

From 2887 abstracts, only eight relevant studies were identified in this review (Figure 2). The characteristic of eight included studies are provided in Table 1. Several populations were involved. Further explanation is discussed in the mechanism of action section.

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Table 1: A summary table of studies discussing the association between epithelial sodium channel (ENaC) and elevated blood pressure

Authors	Year	Type of Study	Subject	Population	Country	Genes responsible for elevated blood pressure	Mechanism of action
Luo et al.[12]	2009	Case-control	Human	Han Chinese descendant	China	rs4149601 variant of NEDD4L	rs4149601 A allele regulates ENaC and elevates blood pressure
Büsst et al.[14]	2007	Cross sectional	Human	Australian white	Australia	*SCNN1G*	Activation of *SCNN1G* increase systolic blood pressure
Guo et al.[21]	2005	Case-control	Human	Mexican American families	United State of America	*SCNN1A*	Activation *SCNN1A* increases insulin receptor thus elevating blood pressure
Chien et al.[11]	2007	Case-control	Human	Chinese	Taiwan	*SCNN1B*	Mutated *SCNN1B* responsible for essential hypertension
Pratt et al.[22]	2002	Cohort	Human	School-age children and young adults Blacks	United State of America	ENaC activity	Reduced ENaC activity lower blood pressure in whites but not blacks
Turner et al.[15]	2012	Case-control	Human	Whites and Blacks with hypertension	United State of America	*SCNN1G*	SCNN1G associated with renin-angiotensin system related to blood pressure
Hannila-Hendelberg et al.[13]	2005	Case-control	Human	Normal and hypertensive Whites	Finland	*SCNN1B* *SCNN1G*	*SCNN1B* and *SCNN1G* associated with renin-angiotensin system related to blood pressure
Jones et al.[16]	2011	Case-control	Human	Normal and hypertensive African	South Africa	*SCNN1B*	R563Q mutation of *SCNN1B* is associated with hypertension.
Persu et al[9]	1998	Case-control	Human	Whites and Africans with hypertension	France	*SCNN1B*	Seven amino acid changes from coding sequences of *SCNN1B* are associated with hypertension in Africans origin.
Baker et al[10]	1998	Case-control	Human	Normal and hypertensive Blacks	London	*SCNN1B*	T594M mutation in *SCNN1B* black people resident in London is associated with hypertension.
Dhanjal et al[17]	2006	Case-control	Human	Black and mixed ancestry women with pre-eclampsia with normotensive pregnant subjects	South Africa	*SCNN1B*	R563Q mutation of *SCNN1B* is associated with pre-eclampsia.

Mechanism of action: Regulation of blood pressure through ENaC subunits.

Genes encoding the beta (β-ENaC) and gamma (γ-ENaC) subunits of epithelial sodium channel are suggested as candidate genes for salt-sensitive forms of hypertension. Gain of function mutations of PPPXY sequence in the cytoplasmic C terminus of either the β- or γ-subunit of ENaC cause Liddle's syndrome, a rare monogenic form of human hypertension associated with low renin activity and low plasma aldosterone level. Liddle's syndrome demonstrates that altered function of ENaC can directly affect blood pressure by increasing ENaC activity [7,8]. Common β-ENaC variants present in increased rate in almost exclusively hypertensive patients of African origin [9,10]. This exclusively shows that the variations of various subunits of ENaC are limited to this ethnic group. There are other well-defined populations i.e.: Chinese [11,12], Finland [13] and Australian [14]. All date points out that there is a need to perform a rigorous genetic-epidemiological studies to identify contribution of ENaC in relation to ethnicity. The data obtained from the screening have suggested that an extensive locus-targeted study on hypertensive family members was identified on chromosome 16q region genes on both the β-ENaC and γ-ENaC which has a significant involved in blood pressure [11,14,15].

Based on a systematic screening conducted by Persu et al., seven amino acid changes at last exon (G589S, T594M, R597H, R624C, E632G) and at exon 8 (G442V, V434M) were identified from the coding sequences of β-ENaC in 525 hypertensive probands mostly of African origin [9]. In a different study in hypertensive patients in Finland, two variants were found in β-ENaC (G589S and il2-17CT) and one in the γ-ENaC variant (V546I) with lowest renin activities and aldosterone concentration [13]. This study revealed that three common genetic variants of β-ENaC and γ-ENaC occur approximately three times more often in patients with hypertension compared to normotensive. Patients carry these variants tended to have suppressed renin

level and aldosterone level, increased urinary potassium excretion, elevated ENaC activity and blood pressure levels. The same variant (G589S) was found in Swedish hypertensive patient [13].

A recent study has also discovered another mutation of the β-ENaC, R568Q, which has strongly associated with hypertension in black population [16]. The new R563Q mutation was identified in 10 of 139 hypertensive patients but in none of the normotensive patients. R563Q mutation of the β-subunit of ENaC was also reported in preeclampsia patients. The frequency of preeclampsia women (7.8%) had R563Q was significantly higher than in the control group 2.6% [17]. According to ethnicity, the frequency of the mutation was significantly higher in black women. The mean renin level was significantly lower in mutation carrier while aldosterone and potassium levels were not different.

Case-control study of black London resident, have shown C-terminus of the β-ENaC subunit (T594M) variant occurs significantly 4 times more frequently in individuals with hypertension than in those without hypertension [10]. This finding suggests that threonine 594 methionine (T594M) point mutation may increase sodium-channel activity and could raise blood pressure in affected people by increasing renal tubular sodium reabsorption. However, the functionality of T594M is questionable because only small but not significant increase Na^+ uptake or Na^+ current was observed in expressed Xenopus oocytes [10].

An *in vitro* study has shown that plasmin urine activate ENaC activity in preeclampsia patients by proteolytic cleavage of the γ-subunit at N terminal. According to Bruns et al., plasmin and prostasin elicit extracellular second hit proteolysis subsequent to intracellular furin to release an inhibitory peptide from γ-ENaC and caused channel activation [4]. This may contribute to an inappropriate renal conservation of Na^+ and water suppression of plasma aldosterone and contribute to hypertension in preeclampsia.

In case control study by Turner et al., 300 whites and 300 blacks with stage 1 to 2 hypertension were treated with candesartan and validated by opposite direction association with response to antihypertensive hydrochlorothiazide for 8 weeks and 6 weeks respectively [15]. 273 polymorphisms were identified; rs11649420 on chromosome 16 in the *SCNN1G* had association with blood pressure (BP) response, involved in mediating renal sodium reabsorption and maintaining blood pressure.

The search was extended in a large population sample (≈3000 people from the Victorian Family Heart Study), the authors examined 26 SNPs found in the *SCNN1G* promoter, intron and exon regions. This finding lead to identification of three polymorphism located at intron 5 and 6 (rs13331086, rs11074553 and rs4299163) that are associated with systolic blood pressure (SBP) in the general Australian white population [14].

Neural precursor cell expressed developmentally down-regulated 4-like (NEDD4L) gene is involved in the development of hypertension by controlling cell surface expression of the kidney epithelial Na^+ channel [18,19]. As NEDDL4 involved in Na^+ homeostasis, mutation in NEDD4L may be responsible for BP phenotype. A potential risk factor for hypertension was identified recently in functional variant of NEDD4L [12]. The authors suggested rs4149601 is associated with the risk of hypertension in Chinese population of hypertensive patients and analyzed the correlation between different genotype with BP response. The study revealed that A allele also exhibit independent sensitive BP response in both SBP and diastolic blood pressure (DBP). Consequently, the variant is very useful as a marker to predict hypertension, orthostatic hypotension and antihypertensive response to hydrochlorothiazide [12].

DISCUSSION

Described is a clinical trial in which black hypertensive individuals who did not fully respond to more traditional therapy were given amiloride, spironolactone, a combination of the two drugs, or placebo. Treatment with either of the active inhibitors of ENaC resulted in a substantial improvement in BP. Therefore, evidence to date is supportive of the concept that an increase in Na^+ transport by ENaC may be a common and requisite component of salt-dependent forms of hypertension. More compelling is the notion that ENaC activity does not fully adjust to an increase in Na^+ reabsorption occurring elsewhere in the nephron, there being overstimulation by inappropriately elevated aldosterone levels. Additional evidence that the maintenance of hypertension can be dependent on ENaC is derived from the observed responses to the treatment of hypertensive individuals with inhibitors of ENaC.

ENaC can be activated direct/indirectly (through protein mediator) to increase blood pressure. Few different genes coded for ENaC (*SCNN1A*, *SCNN1B, SCNN1G*) and protein mediators were involved in the regulation of ENaC (NEDD4L) and hypertension. This protein is important because it assists various cellular functions, from regulation of transcription to intracellular trafficking, as well as protein degradation [20] which involves multiple domains.

Although many genome wide association studies (GWAS) have identified several genetic variants contributing to the elevated blood pressure, but none of them has shown a strong correlation with three subunits of ENaC. Therefore, they are excluded from this systematic review. In result of this, it is difficult to identify a specific gene that responsible in elevating blood pressure through ENaC.

CONCLUSION

Although several molecular variants of ENaC subunits have been identified, there has been no consistent demonstration of an association of any of the variants with hypertension. Therefore, there is a need to conduct more epidemiological studies in other places as mentioned above especially in the South of East Asia.

The literatures concerning genetics influence in human essential hypertension suggests that a number of candidate genes are involved. However, the results often correlated with the origin and places where the study is conducted. Because hypertension is a heterogenous problem with delayed penetrance, the identification of robust intermediate phenotypes with greater heritability and early penetrance would be desirable. Next, by utilizing results from *in vivo* models of hypertension may uncover additional genes to examine. This to confirm which of the genes is responsible for elevating blood pressure. Many pathways mediate blood pressure regulation and there are many population / racial divisions and environmental changes. The concept of gene and environmental factors highlight the importance of the prevalence of hypertension, and it requires further investigation in future studies.

If we can achieve the gene specific for a specific population, pharmacogenomics can utilize the data of ENaC expression and tailor a drug that specifically targets the significant population in controlling symptoms derived from hypertension.

Abbreviations

ENaC: Epithelial sodium channel; β-ENaC: β-subunit of epithelial sodium channel; γ-ENaC: γ-subunit of epithelial sodium channel; T594M: Threonine 594 methionine; BP: Blood pressure; SBP: Systolic blood pressure; NEDD4L: Neural precursor cell expressed developmentally down-regulated 4-like; DBP: Diastolic blood pressure; GWAS: Genome wide association studies

Authors' contributions

All authors contribute in designing the study protocol. NASI and NFAS were responsible for abstract screening, data extraction, analyzing the data and drafting the manuscript. JCH was the technical expert and involved in critical revision of manuscript. AMH performed the search strategy and literature search. SNH was involved in search strategy, research approval and manuscript editing. All authors read and approved the final manuscript.

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Appendix: **Medline search strategy**

Database: Ovid from 1922 to July week 2013

1. hypertension.mp.
2. sodium.mp.
3. blood pressure.mp.
4. kidney.mp.
5. cardiovascular disease.mp.
6. 1 or 2 or 3 or 4 or 5

7. epithelial sodium channel.mp.
8. ion channel.mp.
9. amiloride.mp.
10. kinetics.mp.
11. pathway.mp.
12. mechanism.mp.
13. protein.mp.
14. gene.mp.
15. expression.mp.
16. subunit*.mp.
17. cell line*.mp.
18. animal model*.mp.
19. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18
20. correlation.mp.
21. relationship.mp.
22. 20 or 21
23. channel.mp.
24. epithelium.mp.
25. endothelial.mp.
26. 23 or 24 or 25
27. case control.mp.
28. cross-sectional.mp. [mp=title, abstract, full text, caption text]
29. 27 or 28
30. 6 and 19 and 22 and 26 and 29
31. (cardiovacular adj2 disease).mp.
32. 1 or 2 or 3 or 4 or 31
33. 19 and 22 and 26 and 29 and 32
34. Na+ sodium.mp.
35. K+ Potassium.mp.
36. high blood pressure.mp.
37. Liddle's Syndrome.mp.
38. genes.mp.
39. Nedd4-2.mp.
40. ENaC.mp.
41. aldosterone.mp.
42. insulin.mp.
43. dexamethasone.mp.
44. steroid.mp.
45. hormones.mp.
46. drugs.mp.
47. 1 or 2 or 3 or 4 or 5 or 31 or 36 or 37
48. WNK.mp.
49. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 38 or 39 or 40
50. 23 or 24 or 25 or 34 or 35
51. 41 or 42 or 43 or 44 or 45 or 46
52. 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 48
53. 33 and 52

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