Mendelian hypertension with brachydactyly as a molecular genetic lesson in regulatory physiology

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Luft, Friedrich C., Okan Toka, Hakan R. Toka, Jens Jordan, and Sylvia Bähring. Mendelian hypertension with brachydactyly as a molecular genetic lesson in regulatory physiology. Am J Physiol Regul Integr Comp Physiol 285: R709–R714, 2003; 10.1152/ajpregu.00174.2003.—Mendelian forms of hypertension have delivered a treasure trove of novel genes. To date, the molecular mechanisms of five such syndromes have been largely clarified, including glucocorticoid-remediable aldosteronism, Liddle’s syndrome, apparent mineralocorticoid excess, an activating mutation of the mineralocorticoid receptor, and pseudohypaldosteronism type 2. Each of these conditions features salt sensitivity with increased sodium and volume reabsorption by the kidney and low plasma renin activity. None of the gene loci for these syndromes has been convincingly linked to hypertension in the general population. We are investigating kindreds who have autosomal-dominant hypertension and brachydactyly. Affected persons invariably have both anomalies. The hypertension is severe and results in death at about age 50 years from stroke. The condition resembles essential hypertension, because renin, aldosterone, and norepinephrine responses are normal and no salt sensitivity is present. The response to antihypertensive drugs is general. Another feature is diminished baroreflex sensitivity with markedly impaired blood pressure buffering. Furthermore, the ventrolateral medulla may be compromised in these patients, because neurovascular anomalies are a regular finding. We mapped the gene(s) for this disease to chromosome 12p and narrowed the chromosomal region by studying more affected families. Interestingly, the same locus was recently mapped in Chinese families with essential hypertension. Our 3-centimorgan region contains genes encoding a phosphodiesterase, an ATP-dependent potassium channel, and its regulator the sulfonylurea receptor 2. Screening of the coding regions revealed that none of these candidate genes harbor obvious mutations; however, other genetic mechanisms may nevertheless compromise their function. Our study underscores the importance of regulatory physiology to the understanding of a complex genetic syndrome.

blood pressure regulation; essential hypertension; linkage; association

BILGINTURAN ET AL. (3) described autosomal-dominant hypertension and brachydactyly over 30 years ago. They examined a family on the Black Sea coast of northeastern Turkey. Affected persons had severe hypertension and brachydactyly; the two phenotypes coincide 100%. We revisited this family together with Bilginturan. All affected individuals featured sharply increasing blood pressure with age and died of stroke, both hemorrhagic and thrombotic, generally before age 50 years. Exceptions were affected family members living in Germany whose hypertension was being treated. We confirmed the phenotype and obtained blood samples for genetic studies. We subsequently mapped the gene(s) to chromosome 12p (26). The logarithm of the odds ratio (LOD) score was 9.29, making the odds that this is the responsible gene locus >1,000,000,000:1. The brachydactyly phenotype, the effect of age on mean arterial blood pressure in affected family members, and our initial linkage results are shown in Fig. 1.

We next performed physiological studies on affected individuals in our clinical research center. Duplex studies of the renal arteries, plasma renin activity, aldosterone measurements, and catecholamine determinations were all normal, making a known secondary cause of hypertension unlikely. We performed volume expansion and volume contraction studies (12) and observed that affected persons were not salt sensitive.
Plasma renin activity, aldosterone, and norepinephrine were all suppressed with saline infusion and increased with a low-sodium diet, furosemide administration, and upright posture (27). In the course of these tests, we also observed that affected persons did not feature advanced atherosclerosis and that their funduscopic examination was remarkably benign (13).

Nagai et al. (22) reported a Japanese child with a deletion syndrome on chromosome 12p. This child had numerous severe anomalies, including thoracic malformations. The authors noted that the child had brachydactyly. Figure 2 shows roentgenograms of the Japanese child compared with a Turkish child of similar age with hypertension and brachydactyly. Both children

Fig. 1. Left: hands from an affected and nonaffected person. Middle: increase in mean arterial blood pressure with advancing age. In nonaffected persons, this increase is similar to that observed in most societies. In affected persons, the increase is dramatic with high mortality above age 50 years. The two exceptions were being treated for hypertension. Right: area of linkage from our original study. Microsatellite markers are given. [Adapted from Ref. 26.]

Fig. 2. A: hand from Japanese child with the deletion syndrome. B: hand from an affected Turkish child. Brachydactilies are type E with cone-shaped epiphyses.
feature very similar abnormalities, including type E brachydactyly and cone-shaped epiphyses. We mapped the deletion syndrome and found an overlap of the deleted segment with the linked region in the Turkish family. This finding narrowed our segment by one-half (1). We next embarked on finding more families with the syndrome to narrow this chromosomal region further. A Canadian family had been described earlier (4), and we subsequently identified an American family (30). We have also identified a family from France and a single case of the syndrome from South Africa (unpublished). We sequenced the coding regions of several candidate genes, including a transcription factor, Sox5, and an ATP cassette transporter, Sur2, regulating an ATP-dependent potassium channel, but did not find any mutations.

The Mendelian hypertension syndromes reported by others all featured clues to their mechanisms by their responses to various drugs (21). For instance, glucocorticoid-remediable aldosteronism responds to spironolactone and is promptly abated when glucocorticoids are given. Liddle’s syndrome responds to blockers of the epithelial amiloride-sensitive sodium channel (ENaC). Pseudohypoaldosteronism type 2 responds very nicely to thiazide diuretics. The activating mineralocorticoid receptor mutation syndrome actually gets worse with spironolactone. We therefore launched a randomized, placebo-controlled, crossover trial with 24-h ambulatory blood-pressure measurements of six medication classes with the expectation that important clues would be forthcoming from clinical pharmacology. This endeavor took over 1 year and showed that all medication classes (ACE inhibitor, β-blocker, thiazide diuretic, calcium channel antagonist, α-blocker, α1-agonist) lowered blood pressure on the average of 8 mmHg (25). We found that therapeutically our patients also resembled essential hypertension in that each class of medication was necessary for blood pressure control.

In the course of these studies, we were able to conduct blood pressure and heart rate measurements in a beat-by-beat fashion. These measurements suggested that baroreceptor reflex sensitivity, at least in younger subjects, was impaired compared with nonaffected persons (30). The baroreflex requires an afferent arm with sensors in the great vessels. The afferent fibers travel with the vagus nerve to the brain stem via the dorsal root entry zone, synapse at the ventrolateral medulla, and then project further in a reflex arc that is integrated in the nucleus of the solitary tract before leaving the central nervous system via the sympathetic outflow. Dittmar (8) first demonstrated the importance of structural integrity of the medulla oblongata for cardiovascular functions in 1873. Cushing (6) studied the effect of intracranial pressure elevation mediated by the brain stem on blood pressure and heart rate. Cushing observed that an increased intracranial pressure causes contraction of the splanchnic vessels and also noted that vagotomy augmented the response. He concluded that a sympathetic stimulus was responsible for the increase in peripheral resistance. Brain stem mechanisms contributing to hypertension, which may be involved in neurovascular compression, have been reviewed in detail elsewhere (5, 9, 19). Stimulation of the norepinephrine-containing C1 neurons of the ventrolateral medulla induces the greatest possible pressor response from the central nervous system. Efferents from these C1 neurons reach the sympathetic neurons in the intermediolateral column of the thoracic medulla. Furthermore, there are reciprocal connections between C1 neurons and the nucleus of the solitary tract, which is the first central area processing afferents from the heart and circulation.

Jannetta et al. (14) observed the blood pressures of 51 hypertensive patients who were operated on for trigeminal neuralgia or hemifacial spasm (14). Blood pressure values normalized in 36 of 42 patients after microvascular decompression surgery of the left ventrolateral medulla. Jannetta et al. (15) subsequently performed animal investigations in baboons that had a small balloon implanted into the region of the ventrolateral medulla. This balloon was connected via a catheter to a second balloon in the thoracic aorta. The aortic balloon caused the balloon impinging on the ventrolateral medulla to pulsate. The pulsatile impulse was conducted over days in the baboons and was associated with an increase in blood pressure sufficient to induce an increase in heart size.

Bilginturan et al. (3) had observed tortuous posterior fossa vessels in the course of an angiogram conducted on one of the Turkish patients years earlier. We therefore tested the Turkish family for the presence of looping vessels in the posterior fossa that might impinge on the ventrolateral medulla. Such condition is known as a neurovascular contact syndrome and has been implicated in trigeminal neuralgia (2). We performed magnetic resonance angiographic studies in 15 hypertensive affected and 12 normotensive nonaffected family members. The results showed that all 15 affected individuals studied had evidence for neurovascular contact (24). All had left-sided posterior inferior cerebellar artery or vertebral artery loops contacting the ventrolateral medulla, while six had bilateral neurovascular contact. None of the nonaffected family members showed evidence of neurovascular contact. Linkage analysis for the two traits (hypertension-brachydactyly vs. neurovascular contact) with chromosome 12p marker showed an LOD score of 9.2, making the odds that these two traits are linked to this locus >1,000,000,000:1.

This finding raised the possibility that the hypertension might be the result of morphological anomalies, because we had also observed that affected persons were ~10 cm shorter in stature. We focused our molecular studies on the brachydactyly phenotype. Inherited skeletal anomalies are commonly caused by alterations in transcription factors important during development. We had already focused on the transcription factor Sox5 and had found no mutations in the gene. A longer splice variant of Sox5, so-called long or L-Sox5, renewed our interest. In the mouse, L-Sox5 is ex-
pressed in the “finger” tips during development (20).

Armed with the mouse cDNA, we set about to delineate the intron-exon structure of the human L-Sox5 gene. Because the gene is over 500,000-base pairs long, this endeavor was not trivial. We also searched for single nucleotide polymorphisms (SNPs) that might be useful for linkage-disequilibrium mapping and narrowing the chromosome 12p region further. We identified several SNPs that unfortunately effectively ruled L-Sox5 out of our linkage segment with the exception of the most peripheral 3'-portion of the gene.

To study the notion of abnormal baroreflex regulation in our subjects further, including the possibility that neurovascular contact might be involved, we performed detailed autonomic testing. We measured arterial blood pressure invasively and found that affected persons had orthostatic hypertension that was ameliorated with volume expansion. We found that affected persons responded with blood pressure increases in an exaggerated fashion compared with nonaffected persons or compared with persons with essential hypertension. When trimethaphan was infused to produce complete ganglionic blockade, the responses of our patients were hardly altered, whereas the responses of control subjects were markedly increased. The groups were no longer different (18). These responses are shown in Fig. 3. Baroreflex heart rate responses in contrast were normal. We measured muscle sympathetic nerve activity directly and observed that affected persons did not have an apparent constant increase in sympathetic drive, compared with nonaffected or unrelated persons. As a matter of fact, muscle sympathetic nerve activity and circulating catecholamines were remarkably low. We have since investigated baroreflex buffering of blood pressure further in patients with essential hypertension and with various autonomic disturbances, including primary autonomic failure and multiple systems atrophy (17). In these studies, we learned that baroreflex blood pressure buffering is markedly and similarly impaired in this Mendelian form of hypertension and multiple systems atrophy. With ganglion blockade, the blood pressure buffering capacity is unmasked. We concluded that persons with autosomal-dominant hypertension and brachydactyly have a major defect in baroreflex buffering of blood pressure. Finally, blood pressure remained increased in these subjects after complete ganglionic blockade. This feature suggests that vascular abnormalities may contribute to the hypertension.

The possibility remained that neurovascular contact might be associated with the abnormal baroreflex buffering of blood pressure. Because neurovascular contact can be operated on with reported success (10), we were obligated to find other persons with hypertension and neurovascular contact (16). We screened patients with essential hypertension with magnetic resonance angiography and studied these individuals with measurements of muscle sympathetic nerve activity and with ganglionic blockade. The responses of these persons were different from those we observed in our patients with autosomal-dominant hypertension and brachydactyly. They did not exhibit profoundly diminished baroreflex blood pressure buffering. We have therefore not felt compelled to offer our patients a neurosurgical intervention at this time.

Mendelian syndromes offer clear insight into mechanisms of disease. However, their general relevance to complex genetic disease is not invariable. Our associates performed family studies in China and identified large kindreds in Shijingshan province, a relatively isolated area. The rationale behind studying a relatively isolated population is the belief that fewer genes might be responsible for complex genetic traits in such isolated societies. The hypertension in these Chinese kindreds was carefully studied. Secondary causes of hypertension were specifically excluded. The renin and aldosterone responses were not suggestive of known Mendelian conditions. A total genome scan was conducted in these families, and the hypertensive trait was linked to chromosome 12p with a significant LOD score of 3.4 (11). Concordance with our locus is excellent. These findings, coupled with an earlier observation from an identical-by-descent sib-pair linkage study of dizygotic twins and their parents from our laboratory (23), provide strong evidence for more generalized importance of the chromosome 12p locus to blood pressure regulation and essential hypertension. The primary candidate genes in the region are those encoding phosphodiesterase 3, an ATP-dependent potassium channel Kir6.1, and the sulfonylurea receptor 2.

Because the screening for mutations in candidate genes did not reveal any positive findings, we performed cytogenetic studies to screen for possible chromosomal rearrangements in the linked segment. The link between cytogenetics and Mendelism has received major impetus with the advent of techniques such as interphase fluorescent in situ hybridization (FISH).

![Fig. 3. Blood pressure increases with phenylephrine dose in affected and nonaffected persons sufficient to increase blood pressure by 12.5 mmHg. Affected persons (●) were markedly hypersensitive and exhibited a 12.5-mmHg increase in blood pressure with <19 μg phenylephrine. Complete ganglionic blockade did not alter the response to phenylephrine in the patients. Normal individuals (○) required ~100 μg phenylephrine to exhibit a 12.5-mmHg increase in blood pressure. However, in normal persons, complete ganglion blockade reduced the amount to 10 μg phenylephrine. Data indicate that the patients show almost zero blood pressure buffering. Eliminating autonomic reflexes reduces blood pressure buffering to that degree in normal subjects. [Borrowed from Jordan et al. (18) with permission from Lippincott Williams & Wilkins.]](http://ajpregu.physiology.org/ by 10.220.32.247 on November 8, 2017)
High-resolution chromosome analysis, molecular cytogenetics, and study of the association between specific chromosome rearrangements and single gene disorders have provided a chromosomal basis to a number of Mendelian diseases (7). Deletions and duplications of small regions, usually <3 megabases in size, result in an alteration of normal gene dosage of a number of unrelated genes physically close to each other and are responsible for contiguous gene syndromes. For example, haplinsufficiency is implicated for del 8q24.1 in Langer-Giedion syndrome, del 17p13.3 in Miller-Dieker syndrome, and del 22q11.2 in DiGeorge and velocardiofacial syndromes (7). Another chromosomal mechanism causing Mendelian phenotypes is translocation, which may eventually interrupt a disease gene. Translocation breakpoints are running through a relevant gene, hindering the production of the gene product. Examples include the Rubinstein-Taybi syndrome.

We were intrigued by a recent report in which aromatase, the key enzyme for estrogen biosynthesis in males, exhibited markedly increased function resulting in severe gynecomastia in a father and son, as well as in a third unrelated person (27). Aromatase activity and mRNA levels in fat and skin and whole body aromatization of androstenedione were markedly elevated in all three subjects. The authors studied the 5′-untranslated regions of aromatase mRNA in the patients. They found two novel 5′-untranslated regions. One was a 45-bp sequence that was not detected in aromatase mRNA from skin or fat samples from the unaffected mother of the patient or from four unrelated male controls. The sequence, which is normally found in the mRNA encoded by the FLJ14957 gene, was found in both the father and the son. Another novel 5′-untranslated region of 170 bp was discovered in aromatase mRNA from the third patient, but not in tissue samples from his unaffected brother or four unrelated male controls. This sequence is normally found in the mRNA encoded by the tropomodulin 3 gene. Both genes mapped to the same locus as the aromatase gene on the chromosome 15q21.2–3 region.

The investigators reasoned that in their patients, the aromatase gene was outflanked with novel promoters that activated the gene continuously. With interphase FISH using bacterial artificial chromosome (BAC) probes, they found that inversion rearrangement mutations had occurred in the three affected persons. The mutations probably gave rise to the overexpression of aromatase in the brain and thus to increased local estrogen production.

We believe that a rearrangement could be responsible for our syndrome. First, the diversity of the phenotypes raises the possibility of a contiguous gene syndrome involving perhaps L-SOX5 and other genes in the region. The candidates, such as PDE3, may be exhibiting gain of function. To test this notion, we are preparing BAC probes for interphase FISH. Concomitantly, we will have to test our candidate genes, namely PDE3, Kir6.1, and the sulfonlurea receptor 2.

We envision physiological studies at the systemic and local levels, including forearm blood flow studies. Milrinone, diazoxide, and glybenclamide may serve as pharmacological probes. Finally, buttocks biopsies will be necessary to study mRNA expression of the candidates, including a survey of the 5′-untranslated region. Finally, physiological studies of isolated blood vessels are foreseen. These studies, conducted on the in vivo, in vitro, and molecular level, are the tools that are necessary for today’s studies on regulatory physiology.

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DISCLOSURES

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