Autosomal-Dominant Hypertension With Type E Brachydactyly Is Caused by Rearrangement on the Short Arm of Chromosome 12

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Abstract—We are studying a Turkish family with autosomal-dominant hypertension and brachydactyly; affected persons die of stroke before 50 years of age. With interphase fluorescence in situ hybridization, we found a chromosome 12p deletion, reinsertion, and inversion in affected persons. This finding suggested that the hypertension could be caused by one or more of 3 genes, the ATP-dependent potassium channel Kir6.1, its regulator the sulfonyl urea receptor SUR2, and the phosphodiesterase PDE3A. We further studied 6 affected and 4 nonaffected persons. Buttocks biopsies were done, small vessels were tested on a myograph, and mRNA was extracted. We performed forearm blood flow studies with intrabrachial artery diazoxide, isoproterenol, and milrinone infusions. Systemic pharmacological testing was done with intravenous diazoxide, nitroprusside, and isoproterenol. PDE3A mRNA was high in vessels from 3 affected subjects, but not high in 3 others. The vessels responded similarly to forskolin, with or without glibenclamide, and to cromakalim. However, there was a suggestion that the dilatation after milrinone might be exaggerated. The forearm infusion studies showed no differences in the responses to diazoxide, isoproterenol, or milrinone. Systemically, affected persons showed a greater blood pressure response to diazoxide and nitroprusside, and a greater heart rate response to isoproterenol than nonaffected persons. The results shed doubt on Kir6.1 and SUR2. The differences in PDE3A expression and responses may be the result of hypertension rather than the cause. Although our 3 candidate genes are no longer likely, the rearrangement we describe greatly enhances the perspectives of this project. (Hypertension. 2004; 43[part 2]:471-476.)

Key words: hypertension ■ genetics ■ gene expression

Mendelian hypertension has revealed novel mechanisms, particularly as related to altered renal salt and water reabsorption.1 Autosomal-dominant hypertension with brachydactyly is an exception.2 We mapped the locus to chromosome 12p.3 The renin-angiotensin-aldosterone responses were normal in affected persons, ruling out salt-sensitive hypertension.4 Blood pressure decreased equally well with 5 different drug classes.5 We found some evidence for a possible central mechanism. Affected persons exhibited neurovascular contact from a looping posterior inferior cerebellar artery that may impinge on the brain stem at the area of the ventrolateral medulla.6 Autonomic testing showed that affected subjects had reduced muscle sympathetic nerve activity and low-normal catecholamines; however, their capacity to buffer increases in blood pressure was markedly impaired.7 We studied a Japanese child with type E brachydactyly and a 12p deletion and recruited more families, thereby narrowing our linkage interval.8,9 Because the transcription factor L-Sox5 regulates collagen IIA1 gene expression in the digits of developing mice, we sequenced the entire 500 000 basepair gene and used single nucleotide polymorphisms (SNPs) for mapping. We found that the gene lies just outside our interval.10 We now show an intrachromosomal rearrangement in the 12p linkage interval. The nature of the rearrangement suggested that the genes encoding the ATP-dependent potassium channel Kir6.1, its regulator the sulfonyl urea receptor SUR2, and the phosphodiesterase PDE3A were possible hypertension candidate genes. Loss of function in the potassium channel might cause vasoconstriction. Gain of function in PDE3A might enhance PDE3A expression leading to increased cAMP hydrolyzes that might promote vasoconstriction because of reduced activation of protein kinase A (PKA) and diminished phosphorylation of the myosine light chain kinases.

Received September 30, 2003; first decision October 31, 2003; revision accepted November 26, 2003.

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Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000111808.08715.ec
Methods
All our studies were approved by the internal review board of the Charité and the University of Heidelberg; written informed consent was obtained. The 6 affected and 4 nonaffected Turkish subjects, who all belong to the family we described earlier, were admitted to our Clinical Research Center and were given a standard hospital diet containing ~150-mmol sodium and 80-mmol potassium. They refrained from smoking and consumed no alcohol.

Interphase Fluorescent In Situ Hybridization
We prepared lymphoblastoid cell lines (LCL) and selected five genomic clones prepared as bacterial artificial chromosomes (BAC: CITB library, Research Genetics) or P1-derived artificial chromosomes (PAC; Genome Systems, Inc) spread over the linkage region. The clones were physically mapped and fluorescent probes were prepared. To analyze their order, a series of three-color interphase fluorescence in situ hybridization (FISH) studies were performed. Briefly, air-dried slides from cell lines were pretreated with RNase A and pepsin, denatured in 70% formamide/2x SSC at 75°C, and dehydrated with an ethanol series. We isolated genomic DNA from clones that we labeled by nick translation with either FluoroX (green), or Cy3 (red), or both (yellow), to generate 500-bp fragments. Probes were denatured in formamide containing hybridization solution for 5 minutes at 75°C and hybridized to slides overnight at 37°C. Slides were washed and stained with DAPI and examined under a fluorescence microscope. In assessing interphase cells, we scored only those chromosomes in which all 3 probes were visualized in close alignment with each other. We scored at least 20 chromosomes for each individual.

RNA Extraction and Reverse Transcription Polymerase Chain Reaction
Buttocks biopsies were performed under local anesthesia as described elsewhere. Small vessels were isolated for physiological studies and mRNA extraction. Arteries were homogenized and centrifuged through a QIAshredder column (Qiagen). Total RNA was extracted with the RNeasy Mini Kit (Qiagen). CDNA synthesis was performed using Superscript II (Gibco BRL) and hexanucleotide primers. Quantitative RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) was performed with the TaqMan Universal Master Mix (Applied Biosystems) in a 25-μL reaction. The sequences of primers and probes are available on request.

Intrabrachial Artery Infusions
We tested the hypothesis that affected family members were less sensitive to the vasodilatory effect of isoproterenol because of increased PDE3A activity, compared with controls. We cannulated the brachial artery of the nondominant arm. Intrabrachial arterial pressure and heart rate (ECG) were recorded. Forearm blood flow was determined by strain gauge plethysmography (Filtrass 2001, Domed, Munich, Germany) in both arms. The hand circulation was initiated by a wrist cuff inflated to 20 mm Hg above systolic blood pressure and heart rate (ECG measured heart rate). After a 45-minute baseline, nitroprusside was initiated and increased at 5-minute intervals until heart rate increased 25 bpm or until systolic blood pressure decreased by 20 mm Hg. Then, for other baseline, diazoxide bolus doses at 12.5, 25, 50, and 75 mg were given.

Isolated Vessels
Small arteries (150 to 300 μm) were dissected from buttoc biopsies and were bathed in cold physiological saline solution (PSS) gassed with 5% CO2 in 95% O2. The PSS contained (all in mM): 120 NaCl, 5 KCl, 1.6 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 NaHPO4, 0.02 EDTA, and 5.5 glucose. Two-mm segments were threaded onto two stainless wires and suspended in 5-mL microvascular myograph baths (Danish Myotechnology, Aarhus, Denmark) according to Mulvany and Halpern. The arteries equilibrated for 60 minutes and were then exposed to isomolar 60-mmol/L KCl-containing solution (KPSS) to ensure their viability and contractility. KPSS was similar to PSS except that NaCl was exchanged with equimolar KCl. After PSS washing, the vessels were subjected to one of the following protocols. A cumulative concentration-effect curve to norepinephrine (3×10⁻⁸ to 3×10⁻⁶ M) was generated for each vessel. After tension had stabilized, the vessels were exposed to increasing doses of forskolin (10⁻⁶ to 3×10⁻⁵ M) to increase the intracellular CAMP concentration via activation of adenylate cyclase in the presence or absence of the ATP-dependent K⁺ channel (SUR2) inhibitor glibenclamide (3×10⁻⁴ M) or the ATP-dependent K⁺ channel opener cromakalim (10⁻⁵ to 10⁻⁴ M). In the second set of experiments, vessel rings were contracted by KPSS. After tension had stabilized, the vessels were exposed to the PDE3 inhibitor milrinone (3×10⁻⁷ to 10⁻⁶ M). In all experiments, a separate vessel segment from each individual was simultaneously monitored as control vessel.

Statistical Analysis
We used analysis of variance, repeated measures where indicated, and Bonferroni corrected t tests. We also used regression analysis, multiple stepwise if indicated; probability value <0.05 was considered significant.

Results

Interphase-FISH
We mapped the locus for autosomal-dominant hypertension with brachyactly to a 3.15-Mb segment lying between the markers D12S1650 and SNP rs1899920. The latter marker lies adjacent to the 3′ untranslated region of L-SOX5. We selected 5 BAC/PAC within the segment: 184C8, 96K9, 134P11, 284C17, and 345P1. Clone 184C8 was mapped upstream of the PDE3A gene, clone 96K9 downstream of the Kir6.1/SUR2 genes, clone 134P11 upstream of both genes, and clones 284C17 and 345P1 downstream of L-SOX5 gene. Because we had 3 probe colors (red, yellow, and green), we tested 5 combinations, namely 96K9–284C17–345P1, 96K9–134P11–284C17, and 184C8–96K9–134P11–284C17, and 184C8–134P11–345P1. Nonaffected persons always showed all 5 combinations in the same color order (Table). In combination 1, 96K9–284 to 345P1, the correct order is red-green-yellow (Figure 1). On the left, an affected person has the order red-green-yellow on one chromosome 12p, but the order red-yellow-green on the other. For
combination 4, 184C8–96K9–284C17, the correct order was green-red-yellow. The affected person has green-red-yellow on one chromosome 12p, but red-yellow-green on the other (Figure 1, upper right). Clone 184C8 should reside at the distal portion of the interval, but now resides between 134P11 and 345P1. Clones 345P1 and 284C17 are inverted (Figure 1, lower). Thus, a deletion, reinsertion, and inversion took place. The PDE3A gene lies proximal to clone 184C8 that is deleted but reinserted between 134P11 and 345P1. The ATP-dependent potassium channel and SUR2 are in the vicinity of the uninvolved BAC/PAC. L-SOX5 is now adjacent to clone 284C17 instead of 345P1 in the affected persons.

mRNA Expression of Kir6.1, SUR2, PDE3A, and SOX5 in Arterial Vessels

TaqMan assays showed no difference in the candidate genes Kir6.1, SUR2, and LSOX5 between affected and nonaffected family members (data not shown). The TaqMan experiments showed, on average, a 147-fold higher PDE3A expression in 3 older (43–4 years), affected, severely hypertensive subjects, compared with 3 younger (23±2 years), less hypertensive-affected subjects. The expression in these 3 older subjects was 176-fold higher than in the 4 nonaffected (39±4 years) normotensive family members (Figure 2).

Isolated Vessels

The response to KCl was similar in vessels from affected and nonaffected persons (Figure 3). The force generated by exposure to norepinephrine was not different. In precontracted vessels, the responses to cromakalim, forskolin, or forskolin in the face of glibenclamide were no different. The responses to milrinone were suggestive. We successfully performed experiments in 2 affected persons. Both showed enhanced relaxation to milrinone, compared with 4 nonaffected persons. One of these subjects had high PDE3A expression, whereas one did not.

Pharmacological Testing

The forearm blood flow experiments showed no consistent differences between the groups (Figure 4). The response to intraarterial diazoxide was not increased in affected persons (Figure 4, upper). We also did not find a major difference in the responsiveness to intraarterial isoproterenol (Figure 4, middle) and milrinone infusions between nonaffected and affected family members (Figure 4, lower). The systemic infusions showed greater responses in affected persons (not...
Our major finding is that autosomal-dominant hypertension with brachydactyly in this Turkish family features a chromosome 12p rearrangement. The rearrangement is complex in that a deletion, reinsertion, and inversion have occurred. The area adjacent to the BAC that is involved in the rearrangement is relatively gene poor. There are expressed sequence tags (EST) in the vicinity, but these have not been further characterized. Our 3 candidate genes that we had sequenced earlier did not yield convincing results in either the gene expression studies or in the physiological studies. Our findings raise the likelihood that more than one gene is responsible for the complex phenotype. The importance of our findings is underscored by the fact that this Mendelian hypertension syndrome may be relevant to essential primary hypertension. Gong et al recently found linkage to chromosome 12p in isolated essential hypertension families from China. Their linkage interval overlaps with our linkage interval.

We believed our candidate genes were reasonable investigative targets. Potassium channels have a major function in regulating smooth muscle tone. SUR2 was particularly attractive because that ATP-dependent K channel regulator has been investigated extensively in gene-disrupted mice. Sur2−/− mice feature hypertension and episodic coronary spasm. We had sequenced the gene earlier and had found no mutations. However, the rearrangement raised the possibility of faulty potassium channel regulation. We used the potassium channel openers diazoxide and cromakalim to test the physiology of this system in the patients and their vessels. The vessels were tested with glibenclamide, a SUR2 blocker. However, we found no consistent differences between affected and nonaffected subjects or their vessels. The gene expression studies showed no differences in these candidates. Affected family members were hypersensitive to systemic diazoxide. However, responsiveness in forearm blood flow to intraarterial

![Figure 2](image_url). PDE3A Expression in arterial vessels dissected from buttock biopsies. The relative expression of PDE3A is shown using 18S RNA as the internal standard in a TaqMan assay. Affected family members are demonstrated in filled bars, nonaffected in striped bars. Patient numbers 004, 035, and 013 are aged between 39 to 50 years. Patient numbers 042, 020, and 043 are between 19 and 27 years old, and patient numbers 046, 055, 062, and 015 range between 30 and 48 years. Thus, the affected persons with high PDE3A expression were more hypertensive and older than affected persons with low PDE3A expression.

![Figure 3](image_url). A, Contractile response to 60-mmOL KCl in ring preparations of affected (n=30) and nonaffected individuals (n=24 rings). B, Cumulative concentration-response curves to (3×10−6 to 3×10−5 M) norepinephrine in affected (n=28 rings) and nonaffected (n=25 rings) individuals. C, Opening of KATP channels by cromakalim-induced (10−4 to 10−5) dose-dependent relaxation in ring preparations of affected and nonaffected individuals. D, Increasing intracellular cAMP levels by forskolin (10−8 to 3×10−6 M) induced dose-dependent relaxation in ring preparations of affected (n=6 rings) and nonaffected individuals (n=9 rings). E, Dox-dependent relaxation induced by forskolin (10−5 to 3×10−5 M) in the presence of glibenclamide (KATP channel blocker, 3×10−5 M) in ring preparations of affected (n=6 rings) and nonaffected individuals (n=7 rings). F, Dose-dependent relaxation induced by milrinone (PDE3 inhibitor, 3×10−7 to 10−5) in ring preparations from 2 affected individuals (013 and 043, n=6 rings) and a nonaffected individual (015, n=4 rings). *P<0.05

shown). Affected subjects were highly sensitive to the depressor effect of systemic diazoxide. A cumulative dose of 137.5-mg diazoxide lowered systolic blood pressure 1.8±4 mm Hg in nonaffected and 18±5 mm Hg in affected family members (P=0.03). Affected family members were also more sensitive to nitroprusside. Systemic isoproterenol infusion at a rate of 1 μg/kg/min increased heart rate 16±2.9 bpm in nonaffected and 24±3.3 bpm in affected family members (P<0.05). The blood pressure response was similar in both groups.

**Discussion**

Our major finding is that autosomal-dominant hypertension with brachydactyly in this Turkish family features a chromosome 12p rearrangement. The rearrangement is complex in that a deletion, reinsertion, and inversion have occurred. The area adjacent to the BAC that is involved in the rearrangement is relatively gene poor. There are expressed sequence tags (EST) in the vicinity, but these have not been further characterized. Our 3 candidate genes that we had sequenced earlier did not yield convincing results in either the gene expression studies or in the physiological studies. Our findings raise the likelihood that more than one gene is responsible for the complex phenotype. The importance of our
Perspectives
Delineating the rearrangement has advanced our search even though our 3 candidates failed under scrutiny. Our strategy is to determine the rearrangements in our remaining families, to recruit more families, and thereby determine the aspect of the rearrangement that all families have in common. Southern blotting will follow to show a different pattern at the breakpoint regions between affected and nonaffected subjects. We will explore the EST in the region. We suggest that this Mendelian hypertension syndrome is likely to be relevant to primary hypertension.

Acknowledgments
The present study was supported by grants-in-aid from the Deutsche Forschungsgemeinschaft, the Helmholtz Gesellschaft, and the HELIOS Research Center, Berlin, Germany. We thank Dr Heidemarie Neitzel and Antje Gerlach for transforming lymphocytes of the family members. We thank Dr Rayaz Malik and Dr Thomas Moesta for assisting us in buttock biopsies and Dr Ashley Izzard for assistance in preparation of vascular vessels.

References

Figure 4. Left, Relative changes in forearm blood flow (FBF) with incremental intrabrachial diazoxide infusion in affected (AF) and in nonaffected family members (NAF). Middle, FBF changes with intrabrachial isoproterenol. Right, Response to intrabrachial milrinone infusion. No significant differences were found.


Autosomal-Dominant Hypertension With Type E Brachydactyly Is Caused by Rearrangement on the Short Arm of Chromosome 12

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Hypertension. 2004;43:471-476; originally published online January 5, 2004; doi: 10.1161/01.HYP.0000111808.08715.ec

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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