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Expression Analysis of Macrodactyly Identifies Pleiotrophin Upregulation

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Abstract

Macrodactyly is a rare family of congenital disorders characterized by the diffuse enlargement of 1 or more digits [1–3] (Fig. 1). It is a rare, congenital disease with no familial inheritance pattern, and usually presents as an isolated, non-syndromic condition. While all tissue elements are involved, including fat, skin, nerve, and bone, histopathologic analysis of macrodactyly tissue is usually remarkable for excess mature adipose tissue [3].

We currently possess no modern molecular insight into macrodactyly. No genes or loci have been implicated in the development of this disease. Neither transcriptional nor protein-level characterization of affected tissues is available. There are no cellular or animal models of macrodactyly.

In this study, we performed the first transcriptional profiling of macrodactyly tissue. We found that a developmental cytokine, pleiotrophin (PTN), was significantly overexpressed across all our macrodactyly samples. The mitogenic functions of PTN correlate closely with the clinical characteristics of macrodactyly. PTN thus represents a promising target for further investigation into the etiology of overgrowth phenotypes.

Introduction

Macrodactyly is characterized by the diffuse enlargement of 1 or more digits [1–3] (Fig. 1). It is a rare, congenital disease with no familial inheritance pattern, and usually presents as an isolated, non-syndromic condition. While all tissue elements are involved, including fat, skin, nerve, and bone, histopathologic analysis of macrodactyly tissue is usually remarkable for excess mature adipose tissue [3].

We currently possess no modern molecular insight into macrodactyly. No genes or loci have been implicated in the development of this disease. Neither transcriptional nor protein-level characterization of affected tissues is available. There are no cellular or animal models of macrodactyly.

In this study, we performed the first transcriptional profiling of macrodactyly tissue. We found that a developmental cytokine, pleiotrophin (PTN), was significantly overexpressed across all our macrodactyly samples. As a mitogen that has been implicated in bone, nerve, vascular, and tumor growth, the functions of PTN correlate closely with the clinical characteristics of macrodactyly. PTN thus represents a promising target for further investigation.

Materials and Methods

This Project was reviewed and approved by the Children's Hospital Boston Institutional Review Board, Assurance #07-11-0428 and by the Brigham & Women's Hospital Institutional Review Board, Assurance # FWA0000484. Pediatric patients who underwent elective surgical debulkings of isolated, nonsyndromic macrodactyly were eligible for this study. Additionally, normal abdominal subcutaneous adipose tissue (SAT) samples were obtained from patients undergoing elective surgeries at either Massachusetts General Hospital or Children's Hospital Boston. During the review of this Project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for securing and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data. Written informed consent was obtained from guardians on the behalf of all minors/children participants.

Approximately 5 mg of adipose tissue were harvested from each patient and sectioned into smaller pieces. Samples were either snap frozen in liquid nitrogen and stored at −80°C or fixed in either fresh 4% paraformaldehyde or 10% neutral buffered formalin (Sigma Aldrich) for 4 hours, transferred to phosphate buffered saline and stored in a 4°C refrigerator.

For each patient, RNA was extracted in biological triplicate via submerision in 1 ml of Trizol, mechanical lysis using an RNase free pestle (Kimble Chase Kontes), addition of 200 μl chloroform, and centrifugation (10,000 g, 15 minutes, 4°C). The supernatant was transferred and purified with RNeasy Mini Columns (Qiagen). 1.2 μg RNA was synthesized into complementary DNA (cDNA) with the Superscript III First Strand Synthesis Kit (Invitrogen).

All RNA samples were analyzed for quality using an Agilent 2100 Bioanalyzer. Hybridization to Affymetrix Human Genome U133 Plus 2.0 Arrays and subsequent array processing was done by Asuragen. All data is MIAME compliant and the raw data has been deposited in the Gene Expression Omnibus database (accession number GSE35820). Adult SAT datasets were found using the Expression Omnibus (GEO) [4] and ArrayExpress [5] databases by searching for the terms “adipose” and “fat”, and
restricting the platform to Affymetrix Human Genome U133 Plus 2.0 Arrays.

Raw expression values were analyzed with the Partek Genomics Suite (Partek). Data were normalized using Robust Multiarray Averaging (RMA) with median scaling, quintile normalization, and background correction. The resulting datasets were preprocessed to remove probesets whose minimum fold change (maximum gene expression value divided by the minimum value) was <2, or whose difference between maximum and minimum values was less than 100. Principal component analysis was performed, and linear models were used to identify statistically significant, differentially expressed probe sets. Hierarchical clustering was performed across all samples using the differentially expressed probesets.

Figure 1. Clinical photos from patients undergoing surgical treatment of macrodactyly. (A) 15 month-old boy with macrodactyly involving the thumb, index and middle fingers. As is often seen, there is associated syndactyly between the index and middle fingers and deviation of all affected digits. (B) The same patient during separation and first stage debulking of the digits. The yellow loops are around the digital nerves, which are enlarged. A large volume of overgrown fat and soft-tissue is being removed. (C) 8 year-old girl with macrodactyly isolated to the middle finger. The excess fat and soft-tissue has been removed revealing enlarged digital nerves tagged with yellow loops.

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GEA was performed using FuncAssociate 2.0 (http://llama.mshri.on.ca/funccommlsite/), which uses a Fisher’s exact test to assess enrichment and a resampling approach to correct for multiple hypotheses. For each of the sample populations, a false discovery rate (FDR) of 0.01 was set as the threshold. The differentially expressed probesets were uploaded into FuncAssociate 2.0 as ordered lists. Analysis was performed using the hgnc_symbol namespace, with 1000 permutations for p-value estimation and a p-value cutoff of 0.05. For qPCR, expression levels of PTN were normalized to the housekeeping gene hypoxanthine guanine phosphoribosyl transferase (HPRT) and measured via Quantifast SYBR Green PCR Kit (Qiagen). To minimize the potential impact of any genomic DNA contamination, primers were designed to span multiple exons. Three technical replicates were performed for each sample. Error bars were computed by adding and subtracting 1 unit standard deviation of the delta Ct values from calibrated delta Ct values. P-values were calculated in Microsoft Excel 2007 using 2-tailed heteroscedastic Student’s-t tests.

For immunohistochemical staining, tissues samples fixed in freshly prepared 4% paraformaldehyde were dehydrated using an ethanol gradient, sectioned (5-micron thickness), and mounted on glass slides (Fisher). The sections were rehydrated and antigen retrieval was performed using citric acid buffer (pH 6.0). The sections were blocked using 5% donkey serum (Jackson Labs) with 0.01% Triton, and incubated with goat polyclonal anti-pleiotrophin antibody (1:100 dilution, Abcam ab10849). Bound anti-PTN antibody was visualized using Alexa Flour® 546 donkey anti-goat IgG (H+L) secondary antibody 1:700 (Invitrogen A-11056). Images were captured using a Nikon Eclipse Ti-s.

RNA was isolated from our samples using a DNasey Blood & Tissue Kit (Qiagen). The isolates were analyzed for quality using an Agilent 2100 Bioanalyzer. Sequencing of the promoter and coding regions of PTEN was performed by Polymorphic DNA Technologies.

Results

Between June 2009 and April 2011, 4 pediatric patients (Patients 1 through 4) who underwent elective surgical debulkings of isolated, nonsyndromic macrodactyly participated in the study. Normal abdominal SAT from 2 adult patients (Patients 5 & 6) who underwent elective surgeries at Massachusetts General Hospital between June 2009 and March 2010 was obtained. Normal abdominal SAT was also obtained from 1 pediatric patient (Patient 7) who underwent an elective abdominal procedure at Children’s Hospital Boston.

RNA was isolated from the 4 macrodactyly adipose tissue samples and hybridized to Affymetrix U133A Plus 2.0 microarrays. We sought to compare our samples against publicly available data sets; since macrodactyly tissues are usually identified as “mature adipose tissue” on histopathologic examination, we chose subcutaneous adipose tissue (SAT) datasets as reference data sets. No suitable data was available in the Gene Expression Omnibus (GEO) [4] or ArrayExpress [5] databases for pediatric samples, thus we performed our analysis against 4 adult SAT datasets comprising 345 patients. Datasets GSE135063 [6], GSE157735 [8] were pooled and referred to as the “GSM” pool. Dataset E-TABM-325 [8] was referred to as the “MoPAGE” pool.

To analyze these data, we performed principal component analysis (Fig. 2A). The top 2 vectors accounted for 23.8% and 8.5% of inter-sample variation. This analysis demonstrated clear separation between macrodactyly and SAT samples. We next developed transcriptional profiles of macrodactyly and SAT. We restricted our analysis to probe sets with false-discovery rates (FDR) < 0.01, fold-changes ≥ 1.5, and p-values < 0.05. With these stringent restrictions, we identified 3093 overexpressed and 4202 underexpressed genes. The large number of differentially expressed genes highlights the stark differences between macrodactyly and normal adipose tissue. The 10 features with the highest fold-changes are shown in Table 1. When hierarchical clustering was performed using these 7295 differentially expressed genes, the macrodactyly samples clustered distinctly and distantly from all SAT samples (Fig. 2B).

To better characterize the differentially expressed transcripts, we performed unbiased gene enrichment analysis (GEA) using FuncAssociate 2.0. [9] The 2 tissue types were each enriched for different gene ontology categories (Table 2). In macrodactyly, there was an enrichment of growth factor response (Table S1), extracellular matrix (Table S2), and patterning (Table S3) genes. In contrast, SAT was enriched for classic adipose tissue gene categories such as regulation of fatty acid oxidation and response to insulin (Table 2).

PTN was the most highly overexpressed gene in macrodactyly (34.4-fold overexpression, p-value = 0.00). The developmental cytokine pleiotrophin was present in both the extracellular space and pattern binding GO annotation categories (Tables S2 & S3). To confirm these findings, we performed quantitative real time polymerase chain reaction (qPCR) and found that in macrodactyly, PTN averaged 127.6-fold overexpression (p = 0.049, Fig. 3A).

In vivo, PTN is secreted into the extracellular space where it binds to heparin. To demonstrate that transcriptional upregulation of PTN resulted into protein overproduction, we analyzed sections from adipose tissue from a patient with macrodactyly (Patient 4) and pediatric SAT (Patient 6) tissue for PTN immunoreactivity. We found no PTN staining in pediatric SAT (Fig. 4A). In contrast, macrodactyly sections were marked by widespread punctate expression of PTN (Fig. 4B). This assay did not allow the localization of PTN expression to a specific cell type. We attempted to quantify PTN overexpression by Western blot [10,11] but the small volume of our samples made this technically infeasible. Li et al. reported that deletion of phosphatase and tensin homologue (PTEN) was associated with PTN upregulation [12]. We thus isolated DNA from our macrodactyly samples and sequenced the PTEN promoter and coding regions. No mutations were identified (data not shown).

Discussion

Macroductyly remains a difficult clinical problem, one with an unknown etiology. Modern treatment strategies are strictly surgical and involve either growth-limiting or digit reducing procedures, or, in severe cases, finger amputation [2,3,13]. Prior to this study, no molecular information was available regarding this disease.

Given the absence of molecular data, we elected to first generate transcriptional profiles of macrodactyly tissue. Analysis of this data identified 7295 differentially expressed genes in macrodactyly compared to adult SAT. The candidate genes overexpressed in macroductyly include well-characterized mitogens such as bone-morphogenetic proteins 5 & 7, transforming growth factor beta 3, and Wnt signaling pathway members (WNT2, WNT3A) (Tables S1 & S2). However, the mitogen with the highest fold-change overexpression was pleiotrophin (Table 1).

To characterize our samples at the biological pathway level, we performed gene enrichment analysis. This again underscored the differences between SAT and macroductyly: SAT was enriched for...
Figure 2. Results of principal component analysis (PCA) and hierarchical clustering of gene expression data from macrodactyly samples. (A) PCA of 4 macrodactyly samples (in triplicate) vs. 345 subcutaneous adipose tissue (SAT) samples. The top 2 vectors account 32.3% of intersample variation. Macrodactyly samples cluster distinctly from SAT. (B) Hierarchical clustering of macrodactyly and SAT samples across differentially expressed genes with fold-change $>1.5$, p-value $<0.05$, and false discovery rate $<0.05$. Macrodactyly samples cluster distinctly and distantly from all SAT samples.

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Classic adipose tissue GO categories such as “regulation of catabolic processes” and “response to insulin stimulus” (Table 2). In contrast, macrodactyly tissues demonstrated enrichment of the gene ontology (GO) categories such as “extracellular space” and “pattern binding” (Table 2).

Interestingly, PTN was present in both of these GO categories (Tables S2 & S3). This fact, combined with its rank as the gene with the greatest fold-change overexpression on microarray analysis, led us to perform confirmatory experiments on this candidate gene. At the transcriptional level, qPCR confirmed PTN overexpression in macrodactyly compared to adult subcutaneous SAT, a depot known for its remarkable proliferative capacity (Fig 3A). While PTN was overexpressed in all macrodactyly samples, the degree of overexpression varied greatly between patients, with overexpression levels being lowest in patient 1. There were insufficient samples to correlate PTN overexpression levels with clinical phenotype, but it is possible that lower overexpression corresponds with milder disease. At the protein level, immunohistochemical staining revealed punctate aggregates of PTN in macrodactyly (Fig. 4B). Control pediatric finger adipose tissue from a patient with polydactyly demonstrated no PTN staining (Fig. 4A).

Pleiotrophin is a promising candidate gene for the pathogenesis of macrodactyly because it promotes growth of nearly all the tissues affected by macrodactyly, including nerve, skin, bone, and cartilage. An 18-kDa protein, PTN was the first developmentally regulated cytokine to be discovered [14]. Its amino acid sequence is the most highly conserved between human, bovine, rat, mouse, and chicken cytokines [15]. It possesses 50% sequence homology with midkine [11,15].

Pleiotrophin has been variously referred to as heparin-binding growth-associated molecule (HB-GAM), heparin-binding neurite outgrowth-promoting factor 1 (HBNF1), and osteoblast-stimulating factor 1 (OSF-1). This variety of names reflects its diverse mitogenic functions. In neural cells, PTN is necessary for proper neurite outgrowth and maturation in the central nervous system [14,16,17]. In the peripheral nervous system it promotes nerve regeneration following injury [11]. PTN is highly expressed in vivo in bone and cartilage, and is upregulated in response to mechanical loading [18,19,20]. As an angiogenic factor, PTN supports endothelial cell proliferation [21]. PTN has also been implicated in a number of tumors including glioblastoma [22,23] and breast cancer [24,25], and its overexpression has been reported to cause malignant transformation in several cell lines [15,26]. While one study suggested that PTN inhibits adipogenesis, the findings were indirect and only used an in vitro model [27].

The overlap between the physiologic functions of PTN and the clinical phenotype of macrodactyly is striking. In neural cells, PTN directs nerve growth and regeneration; clinicians have long noted that macrodactyly nerves are unusually large [3,28,29]. PTN is osteogenic and chondrogenic; one of the hallmarks of macrodactyly is persistent bone and joint overgrowth. As a protooncogene, PTN drives
fibroblast, endothelial cell, and epithelial cell growth; all of these soft tissue elements are overgrown in macrodactyly. In many patients, macrodactylyous overgrowth is proportionally patterned among all tissues of the affected region. The means by which this specific, patterned overgrowth occurs is not known.

Table 2. Enriched gene ontology categories in macrodactyly and subcutaneous adipose tissue.

| # of genes | P-adjusted | GO ID     | GO Category                                      |
|------------|------------|-----------|--------------------------------------------------|
| **Upregulated in Macrodactyly** |            |           |                                                  |
| 6          | 0.001      | GO:0071363| cellular response to growth factor stimulus       |
| 6          | 0.039      | GO:0070848| response to growth factor stimulus                |
| 10         | 0.048      | GO:0030199| collagen fibril organization                      |
| 18         | 0.011      | GO:0001501| skeletal system development                       |
| 19         | 0          | GO:0005578| proteinaceous extracellular matrix                 |
| 22         | 0          | GO:0031012| extracellular matrix                              |
| 30         | 0          | GO:0005539| glycosaminoglycan binding                         |
| 34         | 0          | GO:0001871| pattern binding                                   |
| 34         | 0          | GO:0030247| polysaccharide binding                            |
| 41         | 0          | GO:0005615| extracellular space                               |
| 41         | 0          | GO:0009611| response to wounding                              |
| 42         | 0.01       | GO:0008284| positive regulation of cell proliferation         |
| 47         | 0          | GO:0048731| system development                                |
| 48         | 0.008      | GO:2000026| regulation of multicellular organismal development|
| 49         | 0.023      | GO:0005102| receptor binding                                  |
| 51         | 0          | GO:0044421| extracellular region part                         |
| 52         | 0.023      | GO:0009653| anatomical structure morphogenesis                |
| 59         | 0          | GO:0007166| cell surface receptor linked signaling pathway     |
| 77         | 0          | GO:0005576| extracellular region                              |
| **Upregulated in Subcutaneous Adipose Tissue** |            |           |                                                  |
| 3          | 0.002      | GO:0015671| oxygen transport                                  |
| 3          | 0.008      | GO:0005833| hemoglobin complex                                |
| 3          | 0.012      | GO:0015669| gas transport                                     |
| 3          | 0.012      | GO:0005344| oxygen transporter activity                        |
| 5          | 0          | GO:0004556| alpha-amylase activity                            |
| 5          | 0          | GO:0016160| amylase activity                                  |
| 6          | 0.044      | GO:0003823| antigen binding                                   |
| 12         | 0.013      | GO:0003995| acyl-CoA dehydrogenase activity                   |
| 17         | 0          | GO:0046320| regulation of fatty acid oxidation                |
| 24         | 0.023      | GO:0032869| cellular response to insulin stimulus             |
| 36         | 0.004      | GO:0032868| response to insulin stimulus                      |
| 43         | 0          | GO:0001525| angiogenesis                                      |
| 55         | 0.013      | GO:0051056| regulation of small GTPase mediated signal transduction |
| 57         | 0.04       | GO:0035467| negative regulation of signaling pathway          |
| 60         | 0.049      | GO:0006732| coenzyme metabolic process                        |
| 71         | 0.01       | GO:0051270| regulation of cellular component movement         |
| 87         | 0.004      | GO:0009894| regulation of catabolic process                   |
| 96         | 0          | GO:0005083| small GTPase regulator activity                   |
| 123        | 0.004      | GO:0030695| GTPase regulator activity                        |
| 124        | 0.007      | GO:0060589| nucleoside-triphosphatase regulator activity      |
| 197        | 0.015      | GO:0046907| intracellular transport                           |
| 204        | 0.012      | GO:0019899| enzyme binding                                    |
| 222        | 0.04       | GO:0035466| regulation of signaling pathway                   |
| 234        | 0          | GO:0044248| cellular catabolic process                        |

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Figure 3. Confirmation of pleiotrophin (PTN) expression in macrodactyly and the known PTN signaling cascade. (A) Relative expression of PTN in macrodactyly vs. adult subcutaneous adipose tissue as determined by quantitative real time polymerase chain reaction. In macrodactyly, PTN averaged 127.6-fold overexpression (p = 0.049). (B) The PTN signaling cascade and crosstalk with Wnt signalling (from Deuel et al.)14.
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Figure 4. Pleiotrophin (PTN) immunostaining. DAPI = nuclear stain, PTN = antibody staining for pleiotrophin, Merge = composite image merging Brightfield, DAPI, and PTN channels. A) No PTN is seen in pediatric subcutaneous adipose tissue from Patient 7. B) Large PTN aggregates are seen in macrodactyly patient 4.

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One mechanism whereby PTN overexpression might result in macrodactyly was recently suggested by the discovery of an activating AKT1 mutation in Proteus syndrome [30]. This mutation leads to constitutive phosphorylation of residues Ser473 and Thr308 in AKT1. PTN, on the other hand, has been shown to rapidly phosphorylate Ser473 of AKT1 in a dose-dependent manner [31]. This link is particularly intriguing because partial gigantism of the hands and/or feet is a hallmark of Proteus syndrome [32]. Unfortunately, phosphorylation of this specific residue is also consistent with rapid proliferation and thus, the use of this biomarker would not be conclusive as to mechanism of overgrowth. For this reason, testing for phosphorylation of residue Ser473 in AKT1 was not performed.

Little is currently known about the regulation of PTN. While it has been reported that PTEN deletion is associated with PTN upregulation, this is indirect and the direct regulation of PTN stemming from PTEN deletion is unknown [12]. Our microarray data did not demonstrate reduction of PTN levels in macrodactyly (data not shown) and sequencing of the PTN locus in macrodactyly samples yielded no mutations.

This study was limited by the unavailability of normal pediatric finger adipose tissue. Only under rare circumstances would it be ethical to remove a significant amount of tissue from a child’s hand for research purposes. Some pediatric hand conditions, such as polydactyly, are managed by finger amputation and therefore are potential sources of pediatric finger adipose tissue. However, these conditions can be caused by germline mutations and therefore are not strictly normal [33]. Because of these limitations, we believe future research will require in vitro and animal models of macrodactyly. The identification of PTN as the first macrodactyly candidate gene points the way towards the development of critical research tools.

**Author Contributions**

Conceived and designed the experiments: FHL AK FC AKG AT CAC BIL. Performed the experiments: FHL FK FC BIL. Analyzed the data: FHL AK FC AT CAC BIL. Contributed reagents/materials/analysis tools: FHL AG FC BIL. Wrote the paper: FHL FK FC AT BIL.

**References**

1. Ben-Bassat M, Casper J, Kaplan I, Lazar Z (1966) Congenital macrodactyly: A case report with a three-year follow-up. J Bone Joint Surg Br 48: 359–364.

2. McCombe D, Kay SP (n.d.) Macrodactyly. Green’s Operative Hand Surgery, 5th ed.

3. Upton J (2006) Failure of Differentiation and Overgrowth. Plastic Surgery.

4. Plaisier CL, Horvath S, Huertas-Vazquez A, Cruz-Bautista I, Herrera MF, et al. (2009) Expression Analysis Macrodactyly.

5. Plaisier CL, Kytitalo M, Weissglas-Volkov D, Sinsheimer JS, Huertas-Vazquez A, et al. (2009) ArrayExpress update: from an archive of functional genomics experiments to the atlas of gene expression. Nucleic Acids Res 37: D468–472. doi:10.1093/nar/gkn089.

6. Plaisier CL, Kytitalo M, Weissglas-Volkov D, Sinsheimer JS, Huertas-Vazquez A, et al. (2009) Galanin preproprotein is associated with elevated plasma triglycerides. Arterioscler Thromb Vasc Biol 29: 147–152. doi:10.1161/ATVBAHA.108.178533.

7. Plaisier CL, Kytitalo M, Weissglas-Volkov D, Sinsheimer JS, Huertas-Vazquez A, Cruz-Bautista I, Herrera MF, et al. (2009) A systems genetics approach implicates USF1, FADS3, and other causal candidate genes for familial combined hyperlipidemia. PLoS Genet 5: e1000642. doi:10.1371/journal.pgen.1000642.

8. Gene expression omnibus website: Expression data from human adipose tissue (n.d.). Available: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15773. Accessed 2011 April 1.

9. Berria GF, Beaver JE, Cénik C, Tasan M, Roth FP (2009) Next generation software for functional trend analysis. Bioinformatics 25: 3043–3044. doi:10.1093/bioinformatics/btp986.

10. Orr B, Yangoucke G, Grace OC, Smith L, Anderson RA, et al. (2011) Expression of Pleiotrophin in the Prostate is Androgen Regulated and it Functions as an Autocrine Regulator of Mesenchyme and Cancer Associated Fibroblasts and as a Paracrine Regulator of Epithelium. Prostate 71: 955–972. doi:10.1002/pros.2124.

11. Blondet B, Carpentier G, Lafdi F, Courtay J (2005) Pleiotrophin Cellular Localization in Nerve Regeneration after Peripheral Nerve Injury: Journal of Histochemistry & Cytochemistry 53: 971–976. doi:10.1369/jahc.2005.03574.

12. Li G, Hu Y, Hsu Y, Liu M, Freeman D, et al. (2006) PTEN Deletion Leads to Up-regulation of a Secreted Growth Factor Pleiotrophin. Journal of Biological Chemistry 281: 10663–10668. doi:10.1074/jbc.M512592200.

13. Ahlqvist E, Arvidsson S, Sjöqvist L, Breuer I, Hedfalk I, et al. (2001) Gene expression pattern of the macrodactyly candidate gene PTN in cell culture and animal models of macrodactyly in older children and adults. J Hand Surg Am 26: 1010–1019. doi:10.1016/j.jhsa.2004.07.004.

14. Rauvala H (1989) An 18-kd heparin-binding protein of developing brain that is distinct from fibroblast growth factor. EMBO J 8: 2933–2943.

15. Deuel TF, Zhang N, Yeh HJ, Silano-Santiago I, Wang Z-Y (2002) Pleiotrophin: a cytokine with diverse functions and a novel signaling pathway. Arch Biochem Biophys 397: 162–171. doi:10.1006/abbi.2001.2705.

16. Kretschmer PJ, Fairhurst JL, Decker MM, Chan GP, Gluzman Y, et al. (1991) Cloning, characterization and developmental regulation of two members of a novel human gene family of neurite outgrowth-promoting proteins. Growth Factors 5: 99–114.

17. Merenmies J, Rauvala H (1990) Molecular cloning of the 18kDa growth-associated protein of developing brain. J Biol Chem 265: 16721–16724.

18. Imai S, Hino TJ, Henola A, Kurata K, Boki K, et al. (2009) Osteocyte-derived HB-GAM (pleiotrophin) is associated with bone formation and mechanical loading. Bone 44: 765–774. doi:10.1016/j.bone.2009.01.004.

19. Tare RS, Orefice ROC, Clarke NMP, Roach JH (2002) Pleiotrophin/Osteoblast-stimulating factor 1: dissecting its diverse functions in bone formation. J Bone Miner Res 17: 2009–2020. doi:10.1159/00006112.

20. Azizan A, Gau J, Gevindraj P, Tapp H, Neame PJ (2000) Chondromodulin I and pleiotrophin gene expression in bovine cartilage and epiphysis. Matrix Biology 19: 521–531. doi:10.1016/S0945-055X(00)00110-4.

21. Zhang N, Zhong R, Perez-Pinera P, Herradon G, Ezquerra L, et al. (2006) Identification of the angiogenesis signaling domain in pleiotrophin defines a mechanism of the angiogenic switch. Biochem Biophys Res Commun 343: 653–658. doi:10.1016/j.bbrc.2006.03.006.

22. Scrideli CA, Carlotti CG, Okamoto OK, Andrade VS, Cortez MAA, et al. (2000) Gene expression profile analysis of primary glioblastomas and non-neoplastic brain tissue: identification of potential target genes by oligonucleotide microarray and real-time quantitative PCR. J Neurooncol 48: 281–291. doi:10.1017/S105114030007579-4.

23. Lu KV, Jiang KA, Kim KY, Singh D, Erazo EQ, et al. (2005) Differential Induction of Glioblastoma Migration and Growth by Two Forms of Pleiotrophin. Journal of Biological Chemistry 280: 26953–26964. doi:10.1074/jbc.M502614200.

24. Wellstein A, Fang WJ, Khatri A, Lu Y, Swain SS, et al. (1992) A heparin-binding growth factor secreted from breast cancer cells homologous to a developmentally regulated cytokine. J Biol Chem 267: 2382–2387.

25. Rell M, LeJeune S, Scott PA, Fox S, Smith K, et al. (1997) Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor-beta1, platelet-derived endothelial cell growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 57: 963–969.

26. Fang W, Hartmann N, Chow DT, Riegel AT, Wellstein A (1992) Pleiotrophin stimulates fibroblasts and endothelial and epithelial cells and is expressed in human cancer. J Biol Chem 267: 2382–2387.

27. Gu D, Yu B, Zhao C, Ye W, Lu Q, et al. (2007) The effect of pleiotrophon signaling on adipogenesis. FEBS Lett 581: 382–388. doi:10.1016/j.febslet.2006.12.043.

28. Moore BH (n.d.) Macrodactyly and associated peripheral nerve changes. J Bone Joint Surg Am 24: 617–631.

29. Appenzeller O, Kornfeld M (1974) Macrodactyly and localized hypertrophic neuropathy. Neurology 24: 767–771.

**Supporting Information**

**Table S1** Genes present in the “Response to Growth Factor Stimulus (GO:0070848)” gene ontology category.

**Table S2** Genes present in the “Extracellular Space (GO:0005615)” gene ontology category.

**Table S3** Genes present in the “Pattern Binding (GO:0018171)” gene ontology category.
30. Lindhurst MJ, Sapp JC, Teet JK, Johnston JJ, Finn EM, et al. (2011) A Mosaic Activating Mutation in AKT1 Associated with the Proteus Syndrome. N Engl J Med 365: 611–619. doi:10.1056/NEJMoa1104017.

31. Powers C, Aigner A, Stoica GE, McDonnell K, Wellstein A (2002) Pleiotrophin Signaling through Anaplastic Lymphoma Kinase Is Rate-limiting for Glioblastoma Growth. Journal of Biological Chemistry 277: 14153–14158. doi:10.1074/jbc.M112354200.

32. Wiedemann HR, Burgio GR, Aldenhoff P, Konze J, Kaufmann HJ, et al. (1983) The Proteus Syndrome. Partial gigantism of the hands and/or feet, nevi, hemihypertrophy, subcutaneous tumors, macrocephaly or other skull anomalies and possible accelerated growth and visceral affections. Eur J Pediatr 140: 5–12.

33. Upton J, Borad, Loren (n.d.) Molecular basis of human upper extremity disorders. Plastic Surgery. Elsevier Inc., Vol. 8. 20–24.