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Non-Syndromic Brachydactyly Type D and Type E Mapped to 7p15 in Healthy Children and Adults from the Jirel Ethnic Group in Eastern Nepal

Kimberly D. Williams^{1,2}, John Blangero³, Janardan Subedi⁴, Bharat Jha⁵, Thomas Dyer³, John L. VandeBerg^{6,7}, Bradford Towne^{8,9}, and Sarah Williams-Blangero^{3,7}

¹Department of Anthropology, Temple University, Philadelphia, PA

²Department of Pediatrics, Temple University School of Medicine, Philadelphia, PA

³Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX

⁴Department of Sociology and Gerontology, Miami University, Oxford, OH

⁵Tribhuvan University Institute of Medicine, Kathmandu, Nepal

⁶Office of the Chief Scientific Officer, Texas Biomedical Research Institute, San Antonio, TX

⁷Southwest National Primate Research Center, San Antonio, TX 78227

⁸Department of Community Health, Wright State University Boonshoft School of Medicine, Dayton, OH

⁹Department of Pediatrics, Wright State University Boonshoft School of Medicine, Dayton, OH

Abstract

Objectives—There is phenotypic overlap between Brachydactyly Type D (BDD) and Brachydactyly Type E (BDE) that suggests a possible common underlying etiology. We seek to understand the genetic underpinnings of, and relationship between, these skeletal anomalies.

Methods—The Jirel ethnic group of eastern Nepal participates in various genetic epidemiologic studies, including those in which hand-wrist radiographs have been taken to examine skeletal development. 2,130 individuals (969 males; 1,161 females) were phenotyped for BDD/BDE. Of these, 1,722 individuals (773 males; 949 females) were genotyped for 371 STR markers spanning the autosomal genome. Variance components-based linkage analysis was used to conduct a genome-wide linkage scan for QTL influencing the BDD/BDE phenotype.

Results—BDD was present in 3.55%, and BDE was present in 0.39%, of the study sample. Because of the phenotypic overlap between two traits, affecteds of either type were considered as affected by a single combined phenotype (BDD/BDE) having a prevalence of 3.94%. The additive genetic heritability of BDD/BDE was highly significant ($h^2 \pm SE = 0.89 \pm 0.13$; $p = 1.7 \times 10^{-11}$). Significant linkage of BDD/BDE was found to markers on chromosome 7p21-7p14 (peak LOD score = 3.74 at 7p15 between markers D7S493 and D7S516).

Conclusions—Possible positional candidate genes in the one-lod support interval of this QTL include *TWIST* and the *HOXA1-A13* cluster. This is the first study to report significant linkage results for BDD/BDE using a large extended pedigree, and the first to suggest that mutations in *TWIST* and/or the *HOXA1-A13* cluster may contribute to these specific skeletal anomalies.

Keywords

BDD; BDE; linkage; Chromosome 7

Introduction

Brachydactyly of the hand is a condition where phalanges and/or metacarpals are malformed (Poznanski, 1984). The disruptions to growth and development associated with brachydactyly range from minor (e.g., formation of small auxiliary or slightly irregular bones) to severe (e.g., absence of bones). Bell (1951) classified brachydactyly as types A through E based on number, location, and patterning of bone involvement. Understanding the genetic underpinnings of brachydactyly, whether it be syndromic or non-syndromic, major or minor in effects and outcomes, can provide insights into fundamental aspects of skeletal development.

Brachydactyly Type D

Brachydactyly Type D (BDD; OMIM 113200), also known as ‘stub thumb’ or ‘murderer’s thumb,’ affects the distal phalanx of the first digit. An affected distal phalanx is shorter, and the base considerably wider, than a normally developed bone. The trait is easily recognized upon examination of radiographs or by physical examination of an individual’s hand (see Figure 1; see also Williams et al. 2011). There have been few studies of BDD, so little is known about its population prevalences worldwide; however, prevalence rates ranging from 0.41% to 4.0% have been reported (Temtamy and Aglan, 2008).

The earliest published study of BDD examined five generations of a single family with 13 affected individuals. The pattern of inheritance in that family suggested an autosomal dominant mode of transmission (Breitenbach, 1923), and subsequent research found similar results (Thomsen, 1928). This mode of transmission of BDD, however, has been disputed (Stecher, 1957; Williams et al. 2008). Incomplete penetrance (Bell, 1951; Goodman et al. 1965; Gray and Hurt, 1984; Sayles and Jailer, 1934; Stecher, 1957), and phenotyping issues that confuse brachydactyly types (e.g., overlapping phenotype of BDD and BDE), may confound determination of the mode of transmission of BDD.

Brachydactyly Type E

Brachydactyly Type E (BDE; OMIM 113300) is rare (Temtamy and Aglan, 2008). It is characterized by shortened 3rd, 4th, and 5th metacarpals, and sometimes the distal phalanx of the first digit (Poznanski, 1984). Bell (1951) noted that there is considerable variation in the number and pattern of affected metacarpals. Hertzog (1968) suggested multiple subtypes of BDE - BDE1 that involves shortening of only the 4th metacarpal; BDE2 that involves variable combinations of metacarpals and variable combinations of middle and distal phalanges; and BDE3 that involves variable combination of metacarpals but has no phalangeal element involvement. It is not clear, though, that these suggested subtypes represent different developmental disruptions, and there is not enough evidence to support these subcategories (e.g., OMIM does not recognize separate designations for these subtypes). In the study presented here, we define BDE as shortening of metacarpals without involvement of phalangeal elements (with the exception of the distal phalanx of the first digit; see Figure 2 and Figure 3).

BDE has been reported to be an autosomal dominant trait (Bell, 1951; Temtamy and McKusick, 1978). With the exception of our current study of the Jirel population reported here, however, BDE has not been examined in a large-scale population study.

Phenotypic and Genetic Overlap between BDD and BDE

As detailed above, BDD affects the distal phalanx of the first digit alone, while BDE affects variable combinations of metacarpals (most often combinations of the 2nd, 3rd, and 4th digits, but sometimes the distal phalanx of the first digit as well). Although Bell (1951) originally classified BDD and BDE separately, investigators have observed that it is not clear that these two forms of non-syndromic brachydactyly have different etiologies or result from different developmental pathways. Indeed, previous studies of BDE include mention of affected distal phalanges of the first digit (Bell, 1951; Goodman et al. 1965; Hefner, 1924; Stecher, 1957). Similarly, studies of BDD have documented involvement of the metacarpals (e.g., Goodman et al. 1965; Hefner, 1924; Robin et al. 1999; Stecher, 1957).

Given the variability in the phenotypic expression of these types of brachydactyly, it is possible that when these types of skeletal anomalies appear without other developmental anomalies, and when only the skeletal system is affected (i.e., when the brachydactyly is *non-syndromic*), BDD and BDE may represent a single type of skeletal developmental anomaly ranging in presentation from mild (i.e., affecting one or both distal phalanges of the first digit) to severe (i.e., affecting multiple metacarpals *and* the distal phalanx of the first digit). In fact, recent genetic evidence regarding these two traits supports this hypothesis. For example, Johnson et al. (2002) demonstrated that mutations of *HOXD13* lead to BDD and BDE (and see Jamsheer et al. 2012 implicating mutations of *HOXD13* leading to BDE). Studies of BDE in subjects presenting anomalies of other body systems (and thus not representing work on isolated or non-syndromic BDE) have also been conducted. For example, BDE in combination with hypertension was mapped to a region of 12p in a single pedigree (Bahring et al. 2004), and the protein Glypican1 was implicated in a study of a brother and sister whose phenotype included BDE, mental retardation, and other limb anomalies (Syrrou et al. 2002).

Materials and Methods

Study Population

The Jirels are a Tibeto-Burman speaking ethnic group living in the town of Jiri in the Jiri region of the Dolakha District eastern Nepal, 190 km east of Kathmandu. The Jirel population numbers approximately 5,000 individuals, most of whom reside in several villages located within a day's walk from the town of Jiri. The Jirels have been the focus of extensive anthropologic, population genetic, and genetic epidemiologic studies for over 20 years (e.g., (Blangero, 1987; Blangero, 1988; Blangero, 1990; Relethford and Blangero, 1990; Williams-Blangero, 1987; Williams-Blangero, 1989a; Williams-Blangero, 1989b; Williams-Blangero, 1990; Williams-Blangero and Blangero, 1989; Williams-Blangero and Blangero, 1990; Williams-Blangero and Blangero, 1991; Williams-Blangero and Blangero, 1992; Williams-Blangero et al. 1993; Williams-Blangero et al. 1998; Williams-Blangero et al. 1999; Williams-Blangero et al. 2002a; Williams-Blangero et al. 2002b; Williams-Blangero et al., 2004; Williams et al., 2007a; Williams-Blangero et al., 2008a, 2008b; Criscone et al., 2010; Williams-Blangero et al., 2012).

The complete genealogy of the Jirel population has been compiled and is regularly updated. Almost all Jirels trace their ancestry back some 150 years to a founding population of approximately 200 individuals comprised of individuals from nearby Sherpa and Sunwar ethnic groups (Blangero, 1987). Population genetic studies have shown that there has been very little gene flow into the population (< 1% per generation) since the founding event in the mid-1800s (Blangero, 1987). Consanguineous matings are actively avoided and cultural practices minimize the potential for inbreeding (Williams-Blangero 1989a, 1989b, 1990). It has been possible to place all Jirels genotyped to date for whole-genome markers (~ 2,500

individuals) into a single pedigree that contains tens of thousands of pair-wise relationships informative for genetic analyses. The number and type of pair-wise biological relationships represented among the 2,130 Jirels phenotyped for BDD/BDE obtained using the *RELATE* subroutine of the computer package SOLAR (Almasy and Blangero, 1998) are shown in Table 1.

Phenotypic and Genetic Data

The study of brachydactyly in the Jirel population initially grew out of examination of hand-wrist radiographs collected for the study of skeletal maturation under the auspices of the Jiri Growth Study. The Wright State University Institutional Review Board for Human Subjects Research, and the Nepal Health Research Council, approved all informed consent procedures. Informed consent was obtained from each adult subject prior to examination. Informed consent was obtained from at least one parent of each child prior to their examination, and each child assented to examination after the procedures were explained to them using age-appropriate language.

Phenotypic data were collected by examination of the most recently taken left hand-wrist radiograph available from each subject. All radiographs were taken following a standard protocol, including maintenance of a film-to-tube distance of 36 inches. In the course of examining the skeletal maturation of Jirel children from annually-collected hand-wrist radiographs, a high prevalence of brachydactyly type A3 (BDA3) had been noted. The resulting Jiri BDA3 Study (Williams et al. 2005; Williams et al. 2006; Williams et al. 2007a; Williams et al. 2007b; Williams et al. 2007c) focused on the study of non-syndromic BDA3 in the Jirel population. In the course of evaluating radiographs for that study two other types of brachydactyly independent of BDA3 were observed - BDD and BDE. Each of these forms of brachydactyly are more apparent in fully mature individuals, but they are all easily detectable in the early stages of development of the phalanges and metacarpals as well. The prevalence of BDD in the total phenotyped sample of 2,130 individuals is 3.55%, while the prevalence of BDE is 0.39%. Given the phenotypic overlap between BDD and BDE (i.e., involvement of distal phalanx of the first digit), the affecteds of both these types of brachydactyly were considered as a combined single phenotype (BDD/BDE) that has a population prevalence of 3.94%.

Genetic Analyses

A total of 2,130 individuals (969 males, 1,161 females), consisting of 1,376 children aged 3 to 18 years and 754 adult relatives, were phenotyped for BDD/BDE status and were available for heritability/quantitative genetic analyses.

Genotyping Method

Of the 2,130 individuals phenotyped for BDD/BDE status, 1,722 (773 males, 949 females; 904 children, 818 adults) had been typed for highly polymorphic genome-wide short tandem repeat (STR) markers and were thereby available for linkage analysis of BDD/BDE. Each individual was characterized for 371 STRs evenly distributed across the 22 autosomes to generate an ~10-cM genome scan. The markers were typed using the ABI Prism Linkage Mapping Set (version 2), and genotyping was performed using automated sequencers (ABI DNA Sequencer, models 377 and 3100).

Error checking

SimWalk2 (Sobel and Lange, 1996) was used to check for the Mendelian consistency of the genotypic data. SimWalk2 uses Markov chain Monte Carlo and simulated annealing algorithms to determine the probabilities of mistyping for each genotype.

Allele frequency estimation

Allele frequencies were estimated using maximum likelihood techniques implemented in the computer program SOLAR (Almasy and Blangero, 1998). Incorporating pedigree structure in the estimation of allele frequencies is important because estimations of gene frequency can be significantly biased if the relationships among individuals in the sample are not taken into account (Boehnke, 1991). All the elements of a location-specific identity-by-descent (IBD) probability matrix were estimated jointly by use of a Markov chain Monte Carlo method incorporated in the program LOKI (Heath, 1997). Multipoint IBD matrices were then imported into SOLAR for subsequent linkage analyses.

Linkage analysis

A standard likelihood framework for variance components linkage analysis (SOLAR; Almasy and Blangero, 1998) was used for all parameter estimation and testing of hypotheses. The widely used normal liability threshold variance component model for linkage analysis of dichotomous traits (Duggirala et al., 1997; Williams et al., 1999) is implemented in the SOLAR analytic package. This general approach to assessment of dichotomous traits has been shown to recover unbiased estimates of quantitative trait locus (QTL) location, and reliable estimates of effect size even when the principal assumption of the model (i.e., normality on the liability scale) does not hold (Duggirala et al., 1997). Although utilizing very few parameters, variance component-based linkage analysis has been shown to be nearly as efficient as highly parameterized classical penetrance-based linkage analysis when the penetrance model is known exactly (which is rarely the case) (Göring et al., 2001). Thus, variance component-based analysis of dichotomous traits effectively captures all of the linkage information that classical linkage approaches do but without the need to assume and estimate a formal penetrance model. Additionally, the use of a normal liability model for dichotomous traits in extended pedigrees yields superior localization power than that of non-parametric methods such as standard affected sibpair analysis (Williams et al., 2004).

Using the threshold model, we tested the null hypothesis that the additive genetic variance due to the i th QTL equals zero (denoting no linkage) by comparing the likelihood of the restricted model to that of a model in which the variance due to the i th QTL was estimated. The difference between the $2 \log_{10}$ likelihoods provided the logarithm of the odds ratio (LOD) score, which is a measurement of the support for the hypothesis of “linkage” over that of “no linkage” at a particular chromosomal location. The p values were determined using a test statistic that is twice the difference in the $\log_e$ likelihoods of the two models, and that is asymptotically distributed as a $\frac{1}{2}, \frac{1}{2}$ mixture of a χ^2_1 variable with a point mass at zero. Because of the large number of tests required to scan the genome using this linkage procedure, we calculated analytical genome-wide p values (Feingold et al. 1993). All statistical results of linkage are provided with experiment-wide levels of significance.

Results

Heritability of BDD/BDE

In the sample of all 2,130 Jirels phenotyped for BDD and BDE, BDD is significantly heritable ($h^2 \pm \text{SE} = 0.70 \pm 0.13$, $p = 6.3 \times 10^{-9}$). In this same sample, the estimated heritability of BDE maximized to its upper bound of 1. Although this heritability estimate is significantly different from zero, it is imprecise; the lower bound of the 95% confidence interval for the BDE heritability estimate is 0.61. This lack of precision is likely due to the relative rarity of BDE that only appears in 9 individuals. Therefore, the appropriate interpretation is simply that there is substantial evidence for a genetic determination of BDE. In this same study sample of all 2,130 Jirels phenotyped for BDD and BDE, the heritability

of the BDD/BDE phenotype also was significant ($h^2 \pm SE = 0.78 \pm 0.11$, $p = 2.0 \times 10^{-12}$). The heritability of BDD/BDE in the subset of 1,722 individuals phenotyped for BDD/BDE and genotyped for whole genome STR markers is of a very similar high magnitude and also is highly significant ($h^2 \pm SE = 0.89 \pm 0.13$, $p = 1.7 \times 10^{-11}$).

Mode of Transmission of BDD/BDE

The mode of transmission of BDD or BDE was examined in earlier studies by observing the patterning of BDD or BDE affected status among individuals within families. Most of these early studies posited both BDD and BDE to be autosomal dominant traits. Some early studies suggested that BDD and BDE evidence reduced penetrance, presumably because published pedigrees at the time contained patterns of affected/unaffected status inconsistent with a fully penetrant autosomal mode of transmission.

In our study presented here, we used two methods to test the mode of transmission of BDD/BDE in the Jirel population. First, for initial illustrative purposes, from the total of 2,130 individuals phenotyped for presence or absence of BDD/BDE we identified 671 parent-offspring triads with complete phenotypic information (i.e., both parents and at least one child phenotyped for BDD/BDE status). These 671 parent-offspring triads are examined in detail in Table 2 which shows that 34 (5.2%) of 655 children born to BDD/BDE-unaffected parents have BDD/BDE, and that just 5 (22.7%) of 22 children born to parents with either a BDD/BDE-affected father or BDD/BDE-affected mother themselves have BDD/BDE, meaning that 17 (77.3%) of the 22 children born to a BDD/BDE-affected father or mother do not have BDD/BDE. Finally, neither of the 2 children born to parents who were both affected with BDD/BDE is affected with BDD/BDE. These initial results suggest that, at least in the Jirel population, BDD/BDE phenotype transmission is best considered as a complex trait with incomplete penetrance.

Formal complex segregation analysis of BDD/BDE is not feasible in the Jirel pedigree due to its large size and complexity. But, given that BDD/BDE represents a developmental feature that is reasonably hypothesized to be the result of a quantitative threshold process (like most dichotomous characters related to bone (Berry and Berry, 1967; Sjøvold, 1973) we next employed advanced variance decomposition methods using the threshold model incorporated in SOLAR to test for the relative importance of dominance variation contributing to BDD/BDE. It is important to note that this test fully utilizes all pedigree information and is not limited only to the information contained in completely phenotyped parent-offspring triads.

We focused on the study sample of 1,722 individuals phenotyped for presence or absence of BDD/BDE and genotyped for STR markers. We compared a model with only an additive (i.e., co-dominant) component to a model that also includes a component due to dominance effects. We found no statistically significant difference between the likelihoods of the two models ($p = 0.148$). That is, we found no evidence that dominance effects are required to effectively model the BDD/BDE phenotype. The total observed additive genetic heritability of BDD/BDE in the study sample of 1,722 individuals, as mentioned above, was 0.89 ± 0.13 ($p = 1.7 \times 10^{-11}$) in the most parsimonious model (i.e., the additive genetic model with fewer estimated parameters than the more highly parameterized model incorporating a dominance component).

The lack of evidence for dominance effects in this analysis that used all available information makes it very unlikely that patterns of BDD/BDE expression, at least in the Jirel study population, is the result of simple autosomal dominant transmission. Similarly, the less than unit (i.e., 1) heritability also strongly suggests a more complex genetic determination of the trait.

Linkage Analysis of BDD/BDE

A significant linkage of BDD/BDE to markers on chromosome 7p21-7p14 (peak LOD score = 3.74 at 7p15 between markers D7S493 and D7S516) was found (see Figure 4 and Figure 5). This peak occurs at approximately 25.4Mb on chromosome 7. The nominal p-value for this location is 1.5×10^{-5} , and the attendant genome-wide p-value is 0.0051. Thus, the statistical evidence for this localization is highly significant. The 1-LOD support interval for this QTL location covers 28 to 50 cM on the genetic scale, or from approximately 15.2 to 31.2 Mb on the physical scale. In addition, we formally tested for pleiotropic effects of the 7p15 QTL on BDD and BDE separately. Specifically, we tested for coincident linkage at the exact position of the chromosome 7 peak at 42 cM. Both BDD ($p=0.0052$) and BDE ($p=0.0292$) are significantly influenced by the chromosome 7p QTL. This finding is consistent with the hypothesis of pleiotropy, and provides statistical genetic support for combining BDD and BDE into one BDD/BDE phenotype.

Discussion

Syndromic versus Isolated Brachydactyly

Brachydactyly of the hand affects different combinations of metacarpals and phalanges, and often occurs as part of a suite of traits that characterize more serious developmental disorders in which the skeletal system is just one that is affected. Some types of brachydactyly have a significant negative impact on health and quality of life (such as brachydactyly type A1; e.g., Lacombe et al. 2010), while other types of isolated brachydactyly result only in benign developmental disruption of a bone or combination of bones and have few deleterious effects on daily life or well-being. Understanding the genetic underpinnings of any brachydactyly, however, whether it's syndromic or isolated, major or minor in effects and outcomes, provides valuable insights into fundamental aspects of skeletal development.

The 1-LOD support interval around our linkage peak for BDD/BDE encompasses approximately 16 Mb (from 15.2 to 31.2 Mb) on chromosome 7, and contains 273 ENSEMBL-defined genes and 176 RefSeq-defined genes. Within this localization interval there are a number of putative positional candidate genes for BDD/BDE. Because of the documented overlap in the phenotypic expression of BDD and BDE (Bell 1951; Temtamy and McKusick 1978), evidence of a common mutation of the homeobox gene *HOXD13* leading to both non-syndromic BDD and BDE (Johnson et al. 2003), and our work finding significant linkage of BDD/BDE to the genomic region (7p15) containing other homeobox genes (the *HOXA1-13* gene cluster), we hypothesize as well that there is significant genetic overlap between these two types of non-syndromic brachydactyly, possibly due to them sharing a common genetically-mediated developmental pathway.

Positional Candidate Genes

A positional candidate gene in the 1-LOD support interval identified in our linkage analysis of BDD/BDE is *TWIST*. One action of this gene in the model organism *Drosophila* is that it may affect transcription of fibroblast growth factor receptors (Shishido et al. 1993) that are important for differentiation of the limb as they mediate apical ectodermal ridge differentiation. Mutations of *TWIST* are implicated in a number of skeletal dysplasias and syndromes including dysplastic skeletal elements. These include pediatric osteosarcomas (Entz-Werle et al. 2005) and Saethre-Chotzen syndrome (De Heer et al. 2004; el Ghouzzi et al. 1997; Krebs et al. 1997) which includes syndactyly as part of its phenotype. Additionally, a contiguous gene syndrome on 7p resulting in a digit anomaly involves a deletion of *TWIST* and a deletion of the *HOXA* cluster.

A large number of genes are at play during the process of cell differentiation, limb bud formation, limb skeletal formation and ossification, and the maturation/maintenance of the skeleton of the limb and digits (e.g., *FGF*, *SHH*, *BMP-2*, among many others). Key in this process, and perhaps most relevant to the study of brachydactyly, may be the *HOX* genes that play important roles in development/patterning along the anterior-posterior axis. The 5' region of the *HOXA* and *HOXD* gene clusters in particular have demonstrated their very important role in the development of the limb (Zakany and Duboule, 1999). Forelimb defects have been observed in *HOXA-9* and *HOXD-9* mutants due to the expression of these genes in early limb bud formation (Zakany and Duboule, 1999). Mutations in *HOXD-12* (Dolle et al. 1993; Kondo et al. 1996), *HOXD-13* (Davis and Capecchi, 1996; Dolle et al. 1993; Kondo et al. 1996), and *EVX-2* (near *HOXD-13*) (Dolle et al. 1993; Herault et al. 1996) all have an impact on the formation of digits, specifically the length and size of metacarpals and phalanges. In particular, *HOXD-13* has been specifically implicated for BDD and BDE (Johnson et al. 2003). Fromental-Ramain et al. (2003) demonstrated that *HOXA-13* and *HOXD-13* are “indispensable” for the development of the autopod (the most distal portion of the limb), and that single mutations of these genes lead to more dramatic disruption of development of the distal region of the limb (digit size reduction, digit/element absence, and specific element dysplasia: metacarpals/metatarsals, phalanges)). They also concluded that patterning in the distal limb is “strictly dependent” on the function of *HOXA-13* and *HOXD-13*.

Bang et al. (2011) offer the intriguing suggestion that BDD or BDE affected status among rheumatoid arthritis patients may confer a protective advantage resulting in less joint erosion in the brachydactyly affected joints, as well as increased responsiveness to some medications. Rather than simply assuming that this is a product of abnormal articulation, they recall that *HOXD-13* mutations have been linked to BDD and BDE (Johnson et al. 2003), and suggest that these mutations may advantageous in general through inhibition of synovial proliferation. They cite two issues, however, with the argument that brachydactyly confers a significant protective advantage with regard to joint erosion: 1) population prevalences of these brachydactylies are not well known and b) they have not identified a *HOXD* mutation among their patients. Our work provides information about prevalence of this trait in a south Asian population, and posits that *HOXA* mutations lead to the same skeletal dysplasia. Mutations such as these in the *HOX* genes, resulting in isolated (non-syndromic) brachydactyly, may have an as yet undermined advantageous function in limb and digit development. Therefore, within these highly evolutionarily conserved genomic regions, seemingly disadvantageous skeletal dysplasia of the hand (in more severe cases leading to potential reduced grip strength, abnormal muscle attachment, or absence of digits) may persist, walking a fine line between reduced functionality of the hand and significant developmental disorders (syndromic brachydactyly perhaps involving additional genetic mutations). Reasons for this may include protective advantages from: a) early joint fusion and/or b) an as yet incompletely understood physiological advantage which actually protects joints not just from more serious rheumatoid arthritis, but also from ubiquitous osteoarthritis as well. These findings by others have particularly interesting implications given the significant linkage we found in our study presented here for BDD/BDE to markers at 7p15 where the *HOXA1-13* cluster resides.

In light of the findings presented here, we suggest the possibility that BDD and BDE may represent a spectrum of affectedness stemming from a common genetic mutation(s). Future work will continue to explore the genetic architecture of these types of brachydactyly in order to examine this hypothesis and to further inform studies of limb and digit development. Specifically, we aim to identify specific polymorphisms that lead to BDD/BDE, and to define the role *TWIST* and the *HOXA1-13* cluster may play in the expression of these forms of brachydactyly.

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Figure 1.
Example of BDD.

Figure 2.
Example of BDE affecting MC4, MC5, and distal first phalanx.

Figure 3.
Example of BDE affecting MC5 and distal first phalanx

Figure 4.
Genome-wide linkage results for BDD/BDE.

Chromosome 7

Figure 5.
Close-up of significant LOD score on chromosome 7 for BDD/BDE.

Table 1

Major pair-wise relationships among 2,130 members of the Jirel pedigree phenotyped for BDD/BDE.

Relationship	# Related Pairs
Parent -offspring	2,011
Siblings	1,903
Grandparent-grandchild	1,113
Avuncular	3,238
Half Siblings	172
Other Second Degree	16
3rd Degree	8,711
4th Degree	12,096
5th Degree	19,890
6th Degree	13,923
7th Degree	11,772
8th Degree	2,945
9th Degree	367
TOTAL	78,157

Table 2

Numbers of BDD/BDE-affected (+) and BDD/BDE-unaffected (–) children born to –/–, +/-, and +/+ parents.

Parents	Child –	Child +	Total Number	% Affected
–/–	621	34	655	5.2
+/-	17	5	22	22.7
+/+	2	0	2	0