Electronic letter

Mutation analysis of *TBX1* in non-deleted patients with features of DGS/VCFS or isolated cardiovascular defects

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EDITOR—Microdeletions of chromosomal region 22q11.2 (del22q11) have been associated with several genetic disorders, including DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), and conotruncal anomaly face syndrome (CTAFS).¹ The major clinical features associated with del22q11 are conotruncal heart defects, hypoplastic or aplastic thymus and parathyroid glands, facial dysmorphism, and learning difficulties. Many of the structures affected in the del22q11 syndrome are derivatives of the branchial apparatus, which is populated by the rostral neural crest cells. This has led to the hypothesis that haploinsufficiency of a gene(s) within the deleted region disrupts the development of these structures. The majority of patients carry a common ~3 Mb deletion²⁻³ and over 20 genes have been mapped to the deleted region. The phenotypic features seen in association with the 22q11.2 deletion are highly variable, even among family members with the same sized deletion.⁴⁻⁵ Monozygotic twins with discordant phenotypes have also been reported.⁶ Thus, additional factors, such as genetic background and environment, can modify the effect of haploinsufficiency of genes within 22q11.2.

Recent studies have shown that mice heterozygously deleted (Df1/+) for part of the region of mouse chromosome 16 homologous to the DGS/VCFS region of 22q11.2 have heart defects similar to those found in DGS/VCFS patients.⁷ On a different genetic background these mice have been found also to have thymic defects.⁸ Several genes are located within the Df1 deleted region, including *Tbx1*. *TBX1* is a member of the T box gene family of DNA binding transcription factors. T box genes have been shown to play an important role in the regulation of developmental processes.⁹⁻¹⁰ Haploinsufficiency of two T box genes, *TBX3* and *TBX5*, are associated with the human genetic diseases ulnar-mammary syndrome and Holt-Oram syndrome, respectively.¹¹⁻¹³

A targeted deletion of *Tbx1* has been made and heterozygous mice were found to have aortic arch defects similar to those seen in the Df1/+ mice.¹⁴⁻¹⁶ Mice homozygously deleted

for *Tbx1* die before or at birth with all the hallmark features seen in DGS/VCFS, including outflow tract defects of the heart, hypoplasia of the thymus and parathyroid glands, cleft palate, and facial abnormalities.¹⁴ These data strongly suggest human *TBX1* as a candidate gene for many of the key features of DGS/VCFS. Mutation analysis of *TBX1* in patients who have DGS/VCFS phenotypes but no detectable deletion of 22q11 will provide insight into the role of *TBX1* in the aetiology of these disorders.

Methods

We screened 105 patients, who did not have deletions of 22q11.2, for mutations in *TBX1*. Samples were collected from two groups of patients: 40 had clinical features consistent with the diagnosis of DGS/VCFS and 65 had isolated cardiac lesions frequently seen in DGS/VCFS (11 with interrupted aortic arch (IAA) type B, one IAA unspecified, 18 with truncus arteriosus (TA), and 35 with aortic arch anomalies (AAA)). The diagnostic criteria for DGS/VCFS required that a patient have two or more of the major features associated with these disorders: congenital heart defects, immune defects (that is, absent thymus, immunodeficiency, or a history of frequent infections), palatal abnormalities and/or speech difficulties, facial dysmorphism, and a history of learning disabilities. Patients with the conotruncal cardiac defects (TA and IAA) and AAA were included in this study because 22q11 deletions have been found in 50% of patients with IAA, 35% with TA,¹⁷ and 22% with AAA.¹⁸ Further, conotruncal heart defects are the most common heart lesions seen in DGS/VCFS patients. These are also the type of heart defects seen in the mouse models of DGS/VCFS. None of the patients in this study was found to have a 22q11.2 deletion when tested by FISH using the marker N25 (D22S75). Additional FISH analysis to exclude the possibility of a smaller deletion was performed on 23 of the 40 DGS/VCFS patients and 15 of 18 patients with TA. This analysis did not detect any smaller deletions in the patients tested. Further, Southern blot

analysis using a mixture of probes from the *TBX1* gene was performed on 100 of the 105 patients to exclude deletions, insertions, and rearrangements that might not be detected by PCR analysis or FISH. No rearrangements or deletions were detected. Linkage analysis to

ascertain 22q11.2 involvement was not possible owing to the paucity of families with multiple affected members.

The genomic structure of *TBX1* has been previously reported.¹⁹ Two alternatively spliced transcripts, *TBX1A* and *TBX1B* (AF012130 and AF012131, respectively), were found that differed in their terminal exons. However, when the mouse genomic sequence became available, comparison of the human and mouse genomic sequences showed that exon 9A of *TBX1A* and exons 9B and 10 of *TBX1B* are not conserved (fig 1A). Instead, a 1 kb region, 400 bp downstream of exon 8 of *TBX1*, was found that has 67% identity to the corresponding mouse sequence (fig 1A). Analysis of the sequence with GenScan (genes.mit.edu/GENSCAN.html) predicted this conserved region to be an excellent candidate exon. A probe, 9C, was generated from this conserved region and hybridised on a multiple tissue northern blot (16 adult and 4 fetal tissues from Clontech). The expression pattern seen with 9C (data not shown) was identical to that previously published for similar northern blots using a probe from the *TBX1* T box region.¹⁹ A search of the human EST database using the exon 9A, exons 9B and 10, or exon 9C sequences showed over 12 EST matches for exon 9C, but none for the previously characterised terminal exons. RT-PCR with primers designed from exon 9C and RNA from human adult skeletal muscle, adult testis, and fetal lung, tissues that were previously shown to express *TBX1*,¹⁹ confirm the presence of this additional alternatively spliced *TBX1C* transcript (AF373867). The comparison of levels of RT-PCR products using primers from the 3'UTR of *TBX1A*, *TBX1B*, and *TBX1C* show that *TBX1C* is the major transcript.

Primers for mutation analysis were designed using the *TBX1C* sequence. Primers for all nine exons, including exon-intron junction regions, were selected (primer sequences available upon request). For exon 9C, the acceptor splice site, the coding region, plus an additional 30 bp 3' of the stop codon (TAA) were analysed. PCR was performed with genomic DNA from all 105 patients and normal controls. Single stranded conformational polymorphism (SSCP) and/or conformation sensitive gel electrophoresis (CSGE) were used to screen for mutations.^{20–22} Changes seen by SSCP or CSGE were confirmed by sequence analysis.

Results

As shown in table 1, we identified eight common polymorphisms and 10 rare variants. Only one of the common polymorphisms results in an amino acid change (1189C→A, His397Asn). In the mouse, this amino acid residue is a histidine. Of the remaining seven polymorphisms, one is a C→T transversion in the 5'UTR, the second is a base pair substitution in intron 7, and the last five polymorphisms are all single base pair changes in the coding region that do not alter the amino acid sequence. These common SNPs may be useful

Figure 1 (A) Schematic representation of the gene organisation of the alternatively processed transcripts, *TBX1A*, *TBX1B*, and *TBX1C*. All three transcripts have the same sequence organisation from exon 1 to exon 8 and these regions are highly conserved in mouse (indicated by solid boxes). The nucleotide identity between the human and mouse sequences from exons 1–8 ranges from 80% to 91%. However, exon 9A of *TBX1A* and exons 9B and 10 of *TBX1B* are not conserved in mice (represented by unfilled boxes). For *TBX1C*, the first 478 nucleotides of exon 9C, representing the coding region, show 80% identity with the corresponding mouse sequence. In the 3'UTR region of exon 9C, immediately after the stop codon, the identity of a block of 250 bp drops to 19.6% compared to the mouse, shown as shaded boxes. The last 215 nucleotides of exon 9C are highly conserved between mouse and human with 88% identity. (B) Diagram showing distribution of the rare variants relative to the *TBX1* gene structure. The T box domain is represented as the black boxes and the coding region outside the T box domain is shown as striped boxes. The positions of the rare variants are indicated by arrows (the numbers refer to the nucleotide sequence). (C) The conserved non-coding regions (CNSs) between the human and mouse. Comparative sequence alignment was performed using PipMaker (<http://bio.cse.psu.edu>). Six CNSs with 75% or higher identity over at least 100 bp were identified in the intron regions, indicated by arrows.

Table 1 Mutation screening of *TBX1* in 105 patients

Position	Nucleotide change*	Amino acid change	No of patients†	No of controls‡	Patient	Diagnosis	Parents
<i>(A) Rare variants</i>							
Exon 2	39nt 5' to ATG C→T	No	1/105	0/101	p23	DGS	Mother is carrier
Exon 5	576 C→T	192(Arg/Arg)	1/105	0/101	p60	TA	Not available
Exon 7	886 C→A	296(Arg/Arg)	1/105	1/101	p59	IAA typeB	Not available
Exon 9	1049 G→A	350(Gly/Asp)	1/105	0/101	Ap22	AAA	Not available
	1187 C→T	396(Pro/Leu)	1/105	0/100	Ap18	AAA	Not available
	1274-1281delACTATCTC	frameshift	1/105	0/139	p13	DGS	Mother is carrier
	1334-1348del15bp‡	445-449delGYHPH	1/105	0/139	p23	DGS	Father is carrier
	1371-1372insCAC	457-458insHis	1/105	0/139	p22	VCFS	Mother is carrier
	1399-1427dup30bp§	466-476dup10Ala	1/105	0/139	p46	IAA	Not available
	1404-1412delCGCTGCCGC	468-471del3Ala	1/105	0/139¶	p65	DGS/VCFS	Mother is carrier
<i>(B) Common polymorphisms</i>							
Exon 2	75nt 5'to ATG G→C	No	38/105	35/101			
Exon 3	297 G→A	99(Ala/Ala)	11/105	1/11			
Exon 4	420 T→C	140(Phe/Phe)	4/105	2/24			
Exon 5	664 C→T	222(Leu→Leu)	37/105	5/10			
Intron 7	46nt to splice acceptor	No	15/105	1/10			
Exon 8	933 A→G	311(Ala/Ala)	37/105	4/10			
Exon 9	1059 A→G	353(Ala/Ala)	15/105	11/101			
	1189 C→A	397(His/Asn)	37/105	39/100			

*nt1 refers to the first nucleotide of the initiation codon ATG.

†The numbers of patients or controls with polymorphisms or variants are reported for heterozygotes. Complete genotyping was not performed in this study.

‡Deletion of GCTACCACCCGACAG.

§Duplication of GCCGCCGCTGCCGCAGCTGCCGCGGCCGCC.

¶Two normal subjects were found, each with a different 9 bp deletion in the same region.

for association studies. Among the rare variants, we identified three deletions, one insertion, one duplication, and five single nucleotide substitutions (table 1, fig 1B). To determine if these variants were low frequency polymorphisms, we screened over 100 normal controls (>200 chromosomes). Only one of the infrequent changes, the C→A transversion at nucleotide 886, was identified in one of the control samples, giving a frequency of ~0.5%. Interestingly, two control samples had deletions similar to the del(9 bp) at nucleotides 1404-1412. All three 9 bp deletions occurred in a tract of 15 alanines. However, when the sequences were aligned, it was possible to show that each 9 bp deletion occurred in a slightly different region of the alanine repeats. Thus, although the nucleotide sequence in these variants is not identical, the resulting change in the protein sequence is unlikely to be associated with alteration in the protein function. None of the remaining eight rare variants were found in the control samples.

For the rare variants, only two of the five single base pair substitutions are predicted to cause a change in the encoded amino acid. The 1049 G→A changes the Gly at 350 to Asp (G350D) in a patient with AAA. However, the sequence of the mouse at this position is not conserved (the mouse has a single Gly whereas the human has two). The second missense mutation is a C→T transition at nucleotide 1187, resulting in Pro→Leu at amino acid residue 396 (P396L) in a patient with AAA. A proline is found at this position in the mouse. Unfortunately, DNA from the parents of these two patients is not available to clarify whether the changes are de novo. Of the three remaining single base pair substitutions, one is the C→A transversion at position 886 discussed above, one is a silent change (576C→T, R192R), and the last is a C→T transition 39 bp 5' of the presumed start codon. The patient with the -39C→T (p23 in table 1) also has a 15 bp deletion (1334-1348del) that results in an in frame deletion of five amino acids (GYHPH)

starting at codon 445. The five amino acids are highly conserved with 100% identity to mouse and 40% to *Drosophila TBX1* sequence (CAA76529). Analysis of samples from this patient's parents and his unaffected brother showed that he had inherited the 15 bp deletion from his father. The -39C→T change was found in his unaffected mother. Sequence analysis shows that his normal brother has the same two changes, the -39C→T and the del(15 bp). Patient 23 had clinical findings that are consistent with DGS, including absent thymus with partial T cell insufficiency, low parathyroid levels, minor ear anomalies and complex conotruncal heart defect (double outlet right ventricle with pulmonary atresia, VSD, and ASD).

One of the remaining three variants was the result of a small insertion within exon 9C in a patient (p22 in table 1) with a phenotype suggestive of VCFS (cleft palate, dysmorphic facial features, autism with moderate-severe mental retardation, and developmental delay). The patient has a 3 bp insertion (1371-1372insCAC), resulting in an additional histidine within a run of five. The mouse has five histidines in this region but the sequence immediately flanking the histidines is somewhat variable. Family analysis indicated that the 1371-1372insCAC was transmitted to the patient from her unaffected mother. This variant was not found in 139 control samples. In a second patient (p46), we identified a 30 bp in frame duplication (1399-1427dup30), resulting in a tract of 25 alanines instead of the 15 normally seen in this position. The comparative region of the mouse sequence has 10 alanines. DNA from the patient's family is not available and so we are not able to determine whether this duplication is de novo. Analysis of 139 controls did not detect this change in any samples. Finally, we found an 8 bp deletion in a patient with the diagnosis of DGS. The deletion occurs between nucleotides 1274-1281 and is predicted to cause a frameshift at amino acid 425, resulting in the replacement of the

terminal 71 amino acid residues from *TBX1* with 187 amino acids of novel sequence. This patient has the hallmark features of DGS, including facial dysmorphism, IAA with a VSD, hypocalcaemia as a result of hypoparathyroidism, absent thymus with virtually no T cells, and developmental delay. The same 8 bp deletion was also observed in the patient's unaffected mother but not in 139 normal controls.

Discussion

Mutation analysis of our cohort of non-deleted patients did not detect any cases with unambiguous loss of *TBX1* function. Considering the strong experimental evidence for a major role of *TBX1* in the 22q11 deletion syndrome from the deletion studies in the mouse,^{14–16} our findings are somewhat surprising. In contrast to our results, the majority of mutations in *TBX5* in association with Holt-Oram syndrome are predicted to produce haploinsufficiency by nonsense or frameshift mutations within the T box region.^{12–13–23} Similarly, five of the 10 published mutations in *TBX3*, found in patients with ulnar-mammary syndrome, are predicted to disrupt the DNA binding domain.²⁴ It is likely that the aetiology of non-deleted DGS/VCFS patients is heterogeneous. Malformations similar to those seen in the 22q11 deletion syndrome have been associated with prenatal exposure to retinoic acids and ethanol, maternal diabetes, and deletions of the short arm of chromosome 10. Therefore, it is possible that mutations showing clear loss of function were not found in *TBX1* because they are very rare. Similarly, if loss of function of more than one gene within the 22q11 deletion region were necessary to mimic the 22q11 phenotype, then the chance of such an infrequent event occurring would be quite small.

Alternatively, it is possible that the rare variants that are characterised in this report are responsible for the patients' phenotypes. In particular, the 8 bp deletion that results in a frameshift in the terminal exon, the del(GYHPH), and the 30 bp duplication that causes the expansion of the alanine tract, are good candidates for altering *TBX1* function. The unaffected parents of four of the patients were available for analysis and in all cases the variant was present in one of the parents, indicating that the changes are not de novo (table 1). These results would appear to rule against a role for the variant in the patient's phenotype. However, studies in mice show that haploinsufficiency for *Tbx1* does not result in cardiovascular defects in all animals. Rather there is reduced penetrance, ranging from 19 to 50%.^{7–14–16} Further, the percentage of mice with cardiovascular anomalies appears to vary depending on the genetic background.^{8–14} Thus, it is possible that the mutations in human *TBX1* might also show variable penetrance.

Seven of the 10 rare variants occur in the last exon of *TBX1*, outside the DNA binding domain (fig 1B). Based on results of T protein, *TBX2* and *TBX3*, the three proteins that have

- Molecular studies have shown that the majority of patients with DiGeorge syndrome/velocardiofacial syndrome (DGS/VCFS) have a common pathogenesis, deletion of chromosomal region 22q11.2. Recent studies using mouse models suggest that *TBX1*, a gene localised in the common deleted region, is a strong candidate for many of the key features of 22q11 deletion syndromes.
- To investigate the role of *TBX1* in DGS/VCFS further, we performed mutation analysis of this gene in 105 patients, who did not have deletions or rearrangements of 22q11.2 by FISH and Southern blot analysis, but had clinical features consistent with DGS/VCFS or had cardiovascular defects associated with deletion of 22q11.
- We identified eight common polymorphisms and 10 rare variants. Among the rare variants, three are deletions, one is an insertion, one is a duplication, and five are single nucleotide substitutions. One of the rare variants results in a frameshift. In several cases, samples from the parents were available for study and analysis suggests that most rare variants are transmitted to the patient from an unaffected parent.
- Although the experiments in the mouse suggest that *TBX1* may be the key gene for the majority of features of DGS/VCFS, our results indicate that isolated loss of *TBX1* function is unlikely to be a common cause of DGS/VCFS or the associated cardiovascular defects.

been examined, the C-terminal domains appear to be necessary for repression and/or transactivation of transcription.^{25–27} Mutations in the C-terminus distal to the DNA binding domain of *TBX3*, *TBX5*, and *TP1T* have been associated with ulnar-mammary syndrome, Holt-Oram syndrome, and isolated ACTH deficiency, respectively. In these instances, the terminal mutations resulted in phenotypes similar to patients with mutations within the DNA binding domain.^{13–24–28} The expansion of alanine tracts in nuclear proteins, similar to that seen in patient 46, have been associated with four human genetic disorders: synpolydactyly/*HOXD13*,^{29–30} cleidocranial dysplasia/*CBFA1*,³¹ oculopharyngeal muscular dystrophy/*PABP2*,³² and holoprosencephaly/*ZIC2*.³³ The parents of patient 46 were not available for DNA testing and thus we were not able to determine if this change is de novo. Lastly, as has been seen for other genetic disorders,³⁴ it is possible that the single base change variants affect the correct processing of the *TBX1* transcript. Since *TBX1* has restricted expression and is not detectable by RT-PCR in lymphoblastoid cell lines, it will be necessary to develop in vitro assays to determine the effect the rare variants have on the RNA processing and protein function.

In this study, only the exons and splice junctions of *TBX1* were screened. Thus, mutations in the regulatory regions would be missed. In addition to the promoter, there are at least six blocks of sequence greater than 130 bp in the intronic regions of *TBX1* that share 75–93% of similarity to the corresponding mouse sequence (fig 1C). These highly conserved regions may harbour regulatory elements. Mutation screening of these control regions will provide more complete analysis. Finally, although *TBX1* may be a critical gene for the aetiology of DGS/VCFS, the possibility that haploinsufficiency of more than one gene in 22q11.2 is necessary for the expression of the DGS/VCFS phenotype cannot be excluded at present.

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- Emanuel BS, Budarf ML, Scambler PJ. The genetic basis of conotruncal cardiac defects: the chromosome 22q11.2 deletion. In: Harvey R, Rosenthal N, eds. *Heart development*. New York: Academic Press, 1999:463–78.
- Edelmann L, Pandita RK, Morrow BE. Low-copy repeats mediate the common 3-Mb deletion in patients with velo-cardio-facial syndrome. *Am J Hum Genet* 1999;64:1076–86.
- Shaikh TH, Kurahashi H, Saitta SC, O'Hare AM, Hu P, Roe BA, Driscoll DA, McDonald-McGinn DM, Zackai EH, Budarf ML, Emanuel BS. Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum Mol Genet* 2000;9:489–501.
- Cuneo BF, Langman CB, Ilbawi MN, Ramakrishnan V, Cuttiletta A, Driscoll DA. Latent hypoparathyroidism in children with conotruncal cardiac defects. *Circulation* 1996;93:1702–8.
- McDonald-McGinn DM, Tonnesen MK, Laufer-Cahana A, Finucane B, Driscoll DA, Emanuel BS, Zackai EH. Phenotype of the 22q11.2 deletion in individuals identified through an affected relative: cast a wide FISHing net! *Genet Med* 2001;3:23–9.
- Goodship J, Cross I, Scambler P, Burn J. Monozygotic twins with chromosome 22q11 deletion and discordant phenotype. *J Med Genet* 1995;32:7486–8.
- Lindsay EA, Botta A, Jurecic V, Carattini-Revera S, Cheah YC, Resenblatt H, Bradley A, Baldini A. Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature* 1999;401:379–83.
- Lindsay EA, Vitelli F, Pramparo T, Huynh T, Baldini A. Variability and pathogenesis of DiGeorge syndrome in mice. *Am J Hum Genet* 2000;67:10.
- Papaioannou VE, Silver LM. The T-box gene family. *Bioessays* 1998;20:9–19.
- Smith J. T-box genes: what they do and how they do it. *Trends Genet* 1999;15:154–8.
- Bamshad M, Lin RC, Law DJ, Watkins WS, Krakowiak PA, Moore ME, Franceschini P, Lala R, Holmes LB, Gebuhr TC, Bruneau BG, Schinzel A, Seidman JG, Seidman CE, Jorde LB. Mutations in human *TBX3* alter limb, aortic and genital development in ulnar-mammary syndrome. *Nat Genet* 1997;16:311–15.
- Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Soultis J, Grayzel D, Kroumpouzou E, Traill TA, Leblanc-Straceski J, Renault B, Kucherlapati R, Seidman JG, Seidman CE. Mutations in human cause limb and cardiac malformation in Holt-Oram syndrome. *Nat Genet* 1997;15:30–5.
- Li QY, Newbury-Ecob RA, Terrett JA, Wilson DI, Curtis ARJ, Yi CH, Gebuhr T, Bullen PJ, Robson SC, Strachan T, Bonnet D, Lyonnet S, Young ID, Raeburn A, Buckler AJ, Law DJ, Brook JD. Holt-Oram syndrome is caused by mutations in *TBX5*, a member of the *Brachyury* (*T*) gene family. *Nat Genet* 1997;15:21–9.
- Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, *TBX1*. *Nat Genet* 2001;27:286–91.
- Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, Pramparo T, Jurecic V, Ogunrinu G, Sutherland HF, Scambler PJ, Bradley A, Baldini A. *Tbx1* haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* 2001;410:97–101.
- Merscher S, Funke B, Epstein JA, Heyer J, Puech A, Lu MM, Xavier R, Demay MB, Russell RG, Factor S, Tokoyoda K, Jore BS, Lopez M, Pandita RK, Lia M, Carrion D, Xu H, Schorle H, Kobler JB, Scambler P, Wynshaw-Boris A, Skoultschi AI, Morrow BE, Kucherlapati R. *TBX1* is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell* 2001;104:619–29.
- Goldmuntz E, Clark BJ, Mitchell LE, Jawad AF, Cuneo BF, Reed L, McDonald-McGinn D, Chien P, Feuer J, Zackai EH, Emanuel BS, Driscoll DA. Frequency of 22q11 deletions in patients with conotruncal defects. *J Am Coll Cardiol* 1998;32:492–8.
- McElhinney DB, Clark BJ, Weinberg PM, Kenton ML, McDonald-McGinn DM, Driscoll DA, Zackai EH, Goldmuntz E. Chromosome 22q11 deletions in patients with isolated anomalies of aortic arch sidedness or branching. *J Am Coll Cardiol* (in press).
- Chieffo C, Garvey N, Gong W, Roe B, Zhang G, Silver L, Emanuel BS, Budarf ML. Isolation and characterization of a gene from the DiGeorge chromosomal region homologous to the mouse *Tbx1* gene. *Genomics* 1997;43:267–77.
- Li L, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, Qi M, Trask BJ, Kuo WL, Cochran J, Costa T, Pierpont ME, Rand EB, Piccoli DA, Hood L, Spinner NB. Alagille syndrome is caused by mutations in human *Jagged1*, which encodes a ligand for Notch1. *Nat Genet* 1997;16:243–51.
- Ganguly A, Rock MJ, Prockop DJ. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proc Natl Acad Sci USA* 1993;90:10325–9.
- Körkkö J, Annunen S, Pihlajamäe T, Prockop DJ, Ala-Kokko L. Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. *Proc Natl Acad Sci USA* 1998;95:1681–5.
- Basson CT, Huang T, Lin RC, Bachinsky DR, Weremowicz S, Vaglio A, Bruzzone R, Quadrelli R, Lerone M, Romeo G, Silengo M, Pereira A, Krieger J, Mesquita SF, Kamisago M, Morton CC, Pierpont ME, Muller CW, Seidman JG, Seidman CE. Different *TBX5* interactions in heart and limb defined by Holt-Oram syndrome mutations. *Proc Natl Acad Sci USA* 1999;96:2919–24.
- Bamshad M, Le T, Watkins WS, Dixon ME, Kramer BE, Roeder AD, Carey JC, Root S, Schinzel A, Van Maldergem L, Gardner RJM, Lin RC, Seidman CE, Seidman JG, Wallerstein R, Moran E, Sutphen R, Campbell CE, Jorde LB. The spectrum of mutations in *TBX3*: genotype/phenotype relationship in ulnar-mammary syndrome. *Am J Hum Genet* 1999;64:1550–62.
- Kispert A, Koschorz B, Herrmann BG. The T protein encoded by *Brachyury* is a tissue-specific transcription factor. *EMBO J* 1995;14:4763–72.
- He M, Wen L, Campbell CE, Wu JY, Rao Y. Transcription repression by *Xenopus* ET and its human ortholog *TBX3*, a gene involved in ulnar-mammary syndrome. *Proc Natl Acad Sci USA* 1999;96:10212–17.
- Sinha S, Abraham S, Gronostajski RM, Campbell CE. Differential DNA binding and transcription modulation by three T-box proteins, T, *TBX1* and *TBX2*. *Gene* 2000;258:15–29.
- Lamolet B, Pulichino A, Lamonerie T, Gauthier Y, Brue T, Enjalbert A, Drouin J. A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. *Cell* 2001;104:849–59.
- Muragaki Y, Mundlos S, Upton J, Olsen BR. Altered growth and branching patterns in synpolydactyly caused by mutations in *HOXD13*. *Science* 1996;272:548–51.
- Akarsu AN, Stoilov I, Yilmaz E, Sayli BS, Sarfarazi M. Genomic structure of *HOXD13* gene: a nine polyaniline duplication causes synpolydactyly in two unrelated families. *Hum Mol Genet* 1996;5:945–52.
- Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR. Mutations involving the transcription factor *CBFA1* cause cleidocranial dysplasia. *Cell* 1997;89:773–9.
- Brais B, Bouchard JP, Xie YG, Rochefort DL, Chretien N, Tome FM, Lafreniere RG, Rommens JM, Uyama E, Nohira O, Blumen S, Korczyn AD, Heutink P, Mathieu J, Durancieu A, Codere F, Fardeau M, Rouleau GA, Korczyn AD. Short GCG expansions in the *PABP2* gene cause oculopharyngeal muscular dystrophy. *Nat Genet* 1998;18:164–7.
- Brown LY, Odent S, David V, Blayau M, Dubourg C, Apacik C, Delgado MA, Hall BD, Reynolds JF, Sommer A, Wiczorek D, Brown SA, Muenke M. Holoprosencephaly due to mutations in *ZIC2*: alanine tract expansion mutations may be caused by parental somatic recombination. *Hum Mol Genet* 2001;10:791–6.
- Cooper TA, Mattox W. The regulation of splice-site selection, and its role in human disease. *Am J Hum Genet* 1997;61:259–66.