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Mutations in the *CHD7* Gene: The Experience of a Commercial Laboratory

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CHARGE syndrome is an autosomal dominant multisystem disorder caused by mutation in the *CHD7* gene, encoding chromodomain helicase DNA-binding protein 7. Molecular diagnostic testing for *CHD7* mutation has been available in a clinical setting since 2005. We report here the results from the first 642 unrelated proband samples submitted for testing. Thirty-two percent ($n = 203$) of patient samples had a heterozygous pathogenic variant identified. The lower mutation rate than that published for well-characterized clinical samples is likely due to referral bias, as samples submitted for clinical testing may be for "rule-out" diagnoses, rather than solely to confirm clinical suspicion. We identified 159 unique pathogenic mutations, and of these, 134 mutations were each seen in a single individual and 25 mutations were found in two to five individuals ($n = 69$). Of the 203 mutations, only 9 were missense, with 107 nonsense, 69 frameshift, and 15 splice-site mutations likely leading to haploinsufficiency at the cellular level. An additional 72 variations identified in the 642 tested samples (11%) were considered to have unknown clinical significance. Copy number changes (deletion/duplication of the entire gene or one/several exons) were found to account for a very small number of cases ($n = 3$). This cohort represents the largest CHARGE syndrome sample size to date and is intended to serve as a resource for clinicians, genetic counselors, researchers, and other diagnostic laboratories.

Introduction

CHARGE syndrome is an autosomal dominant condition characterized by a nonrandom cluster of congenital anomalies including coloboma of the eye, heart defects, choanal atresia, retarded growth, genital abnormalities, and inner and outer ear anomalies, as well as hyposmia and other cranial nerve abnormalities (Pagon *et al.*, 1981; Blake *et al.*, 1998). Numerous less-common features, including abnormal kidney, cleft lip/palate, and tracheoesophageal fistula, have also been reported. The clinical presentation of CHARGE syndrome can be highly variable (Zentner *et al.*, 2010). Estimates of the incidence of CHARGE syndrome range from 1:8500 to 1:12,000 (Issekutz *et al.*, 2005) (Kallen *et al.*, 1999).

De novo mutations in the gene encoding chromodomain helicase DNA-binding protein 7 (*CHD7*) are the major cause of CHARGE syndrome (Vissers *et al.*, 2004). DNA sequencing detects *CHD7* mutations in ~58%–64% of patients clinically diagnosed with CHARGE syndrome (Vissers *et al.*, 2004; Jongmans *et al.*, 2006; Lalani *et al.*, 2006). Of the *CHD7* mutations reported thus far, ~70% are nonsense or frameshift, 6%–13% are missense, and 7%–15% are splice site mutations (Vissers *et al.*, 2004; Felix *et al.*, 2006; Jongmans *et al.*, 2006; Lalani *et al.*, 2006; Sanlaville *et al.*, 2006; Aramaki *et al.*, 2007; Vuorela *et al.*, 2007; Asakura *et al.*, 2008; Bergman *et al.*, 2008; Gennery *et al.*, 2008; Wincent *et al.*, 2008). Partial and whole gene deletions or duplications are rare, accounting for 3%–4% of pathogenic *CHD7* mutations (Aramaki *et al.*, 2006; Vuorela *et al.*, 2007; Bergman *et al.*, 2008; Wincent *et al.*, 2008). Although germline transmission of *CHD7* mutation has been reported (Pauli *et al.*, 2009), the majority of mutations arise *de novo*.

GeneDx is a Clinical Laboratory Improvement Amendments-certified commercial laboratory that specializes in genetic testing for over 250 rare genetic disorders. Clinical testing of the *CHD7* gene at GeneDx has been available since 2005 and is performed on patient specimens using sequence analysis and, when indicated or desired, copy number analysis. The clinical utility of *CHD7* gene analysis is to confirm a clinical diagnosis of CHARGE syndrome or to resolve a differential diagnosis that may include diseases with similar or overlapping clinical presentations, such as Kallmann syndrome, 22q11 deletion syndrome, VACTERL association, and retinoic embryopathy. In addition, patients presenting with one or two of the clinical features of CHARGE syndrome, such as coloboma or choanal atresia, may also be referred for *CHD7* testing as these patients could have an unusual presentation of the disease.

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Here we present the results of 642 unrelated patient samples submitted to GeneDx for *CHD7* mutation analysis. Based on recommendations by the American College of Medical Genetics (Richards *et al.*, 2008) for designating a variant as pathogenic, 203 of the 642 samples tested (32%) contained a variant in the *CHD7* gene that was considered pathogenic. This differs from other reports in the literature of 58%–64% positive rate of *CHD7* sequencing in CHARGE patients, reflecting the frequent lack of detailed clinical data provided with samples submitted to a clinical service laboratory. Twenty five of the 159 unique mutations (16%) were observed more than once, suggesting the presence of mutational hotspots within *CHD7*. We could not determine the clinical significance of additional 72 variants because of lack of available parental samples, clinical information, or functional data.

Methods

Criteria for diagnosis are defined by physicians and genetic counselors and are not provided to us. Genomic DNA was purified from buccal swabs or peripheral blood lymphocytes by standard methods. The protein-encoding exons of the *CHD7* gene, exons 2–38, were amplified using oligonucleotide primers targeting intronic sequence flanking *CHD7* exons under standard polymerase chain reaction conditions and sequenced bidirectionally by capillary sequencing on an ABI3730, using primers designed and optimized by the clinical laboratory. To help in identifying polymorphisms, synonymous and nonsynonymous variants were examined for conservation with the zebrafish CHD7-like protein (XP_697956). The zebrafish sequence was chosen for this purpose because it aligns well with the human sequence, yet it has more divergence from human CHD7 than other available sequences. Human and mouse CHD7 proteins are 97.1% identical, and human and chicken have 91.9% identity, whereas human and zebrafish CHD7s have 64.2% identity.

Testing for a *CHD7* exon deletion or duplication is now performed by the laboratory upon request, using either multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA kit P201-B1; MRC-Holland) or exon-level resolution oligonucleotide array comparative genomic hybridization (ExonArray). A proprietary CopyDx quantitative polymerase chain reaction method is used to confirm whole or partial gene deletions or duplications. We tested 11 samples by MLPA, 8 by exon array, and 4 by CopyDx after DNA sequencing showed no obvious disease-causing mutation and few or no heterozygous polymorphisms.

Results

Six hundred forty-two patients were referred by physicians and other authorized providers to GeneDx for clinical genetic testing of the *CHD7* gene. For each specimen, the entire protein coding sequence of the *CHD7* gene, along with intron sequence flanking each exon, was analyzed by DNA sequencing. Variants predicted to introduce premature stop codons or cause frameshifts were considered pathogenic. Variants involving the canonical splice donor–acceptor pair (GT-AG) were also considered pathogenic, in keeping with American College of Medical Genetics guidelines (Richards *et al.*, 2008). Missense changes or other putative splicing changes were considered pathogenic if proven *de novo* by testing both parents for its absence, or if the change was identical to a previously reported *de novo* disease-associated mutation. Heterozygous polymorphisms were recorded to document the presence of two alleles.

Table 1. Summary of Results for 642 DNA Samples Analyzed

Samples	642
No disease-causing mutation identified by DNA sequencing	401
No heterozygous polymorphism	76 → one whole gene deletion one exon 2 deletion one exon 3 duplication
With heterozygous polymorphisms	325
Disease-causing mutation identified by DNA sequencing	200; 156 unique
Nonsense mutations	107; 68 unique
Frameshift mutations	69; 66 unique
Splicing mutations	15; all unique
Missense mutations	9; 7 unique
Mutations of undetermined clinical significance	72
Missense (nonsynonymous)	36 (excluding 3 putative splice variants); 33 unique
Inframe residue insertion/deletion	3; all unique
Potential splice:	
Intronic	17; 9 unique
Exonic, nonsynonymous	3; all unique
Exonic, synonymous	13; all unique

We identified pathogenic *CHD7* mutations in 203 (32%) patient samples. Two hundred of 203 mutations involved small, mostly single base changes, which were detected by DNA sequencing. These include 107 nonsense mutations, 69 frameshift mutations, 15 splicing mutations, and 9 missense mutations (Tables 1 and 2). One nonsense mutation was identified in the blood sample from a parent who was presumed to be mosaic. Two of the nine mutations classified as missense are located in the last bases of exons 8 and 17. These two mutations could affect splicing, as mutations at the end of exons are reported to inhibit the ability of the exon to be recognized by splicing factors (Talerico and Berget, 1990). We observed 25 different mutations more than once, including 7 not previously reported (Vissers *et al.*, 2004; Felix *et al.*, 2006; Jongmans *et al.*, 2006; Lalani *et al.*, 2006; Writzl *et al.*, 2007; Gennery *et al.*, 2008) (Table 2). Of these 25 mutations, 20 are nonsense, 3 are frameshift, and 2 are missense. The mutations we have identified are distributed throughout the coding region and do not appear to be preferentially located within regions corresponding to functional domains. The locations of the single-base mutations in the *CHD7* gene and corresponding protein are shown in Figure 1.

Twenty-three specimens were further analyzed for copy number changes, using MLPA, CopyDx, or exon-level CGHarray. Of these cases, one duplication of exon 3, one whole gene deletion, and one deletion of exon 2 were identified.

A number of variants were identified in patient specimens that were novel or eluded classification (Table 3a). In most cases ($n = 54$) this is due to the lack of available parent samples.

Table 2. CHARGE-Causing Mutations Identified

Accession no.	*Type*	*DNA mutation*[a]	*Protein mutation*	*Parental testing*	*Previously reported*
052044	f	c.191_194delCAAA	p.T64fsX65		
063616	x	c.253C>T	p.Q85X		
073716	f	c.285delG	p.G95fsX210		
801921	x	c.334C>T	p.Q112X		4
808037	x	c.435G>A	p.W145X		4
060362	x	**c.469C>T**	**p.R157X**		1, 3, inherited in 7
900517	x	**c.469C>T**	**p.R157X**		1, 3, inherited in 7
051962	x	c.502C>T	p.Q168X		
076598	x	c.511C>T	p.Q171X		
062461	x	c.562C>T	p.Q188X		
807028	x	c.601C>T	p.Q201X		
054032	f	c.729delC	p.P243fsX304	*De novo*	
060395	f	**c.780delC**	**p.P260fsX304**		
808774	f	**c.780delC**	**p.P260fsX304**		
076601	f	c.865delA	p.T289fsX304		
062556	x	c.889C>T	p.Q297X		
051582	f	c.900dupC	p.S301fsX317		
071214	x	**c.934C>T**	**p.R312X**	*De novo*	9
074583	x	**c.934C>T**	**p.R312X**		9
804090	x	**c.934C>T**	**p.R312X**		9
806016	x	**c.934C>T**	**p.R312X**		9
808274	x	**c.934C>T**	**p.R312X**		9
801830	x	c.939T>G	p.Y313X		
053758	x	c.1024C>T	p.Q342X		
805081	f	c.1079_1095del17	p.G360fsX368		
060959	f	c.1095_1096insTC	p.P366fsX377		*De novo* in 4
903850	f	c.1140_1143dupTATG	p.H382fsX405		
801859	x	c.1153C>T	p.Q385X		
074683	f	c.1310dupA	p.H437fsX574	One parent excluded	
060219	x	c.1312C>T	p.Q438X		
802161	f	c.1319delC	p.P440fsX462		
077524	x	**c.1366C>T**	**p.Q456X**		
902216	x	**c.1366C>T**	**p.Q456X**		
052350	f	c.1374_1375delTC	p.S458fsX573		
051457	x	**c.1480C>T**	**p.R494X**		*De novo* in 4 and 11
061371	x	**c.1480C>T**	**p.R494X**		*De novo* in 4 and 11
074832	x	**c.1480C>T**	**p.R494X**		*De novo* in 4 and 11
803442	x	**c.1480C>T**	**p.R494X**		*De novo* in 4 and 11
902758	x	**c.1480C>T**	**p.R494X**		*De novo* in 4 and 11
812180	f	c.1488dupA	p.P497fsX574		
808384	x	c.1510C>T	p.Q504X		
802325	f	c.1544delC	p.P515fsX563		
811230	x	c.1576C>T	p.Q526X		
802993	f	c.1610_1611insA	p.W537fsX537		
061801	f	c.1689dupA	p.E564fsX574		
811915	f	c.1730dupA	p.N577fsX584		
076142	f	c.1818_1819insAT	p.V607fsX608		
075651	f	c.1925delA	p.K642fsX710		
065234	f	c.2034delA	p.K678fsX710		
807136	s	c.2096+2T>C	IVS3+2T>C	One parent excluded	
053440	f	c.2180delT	p.L727fsX727		
070341	f	c.2244_2245delAC	p.R748fsX760		
061959	x	c.2311G>T	p.E771X		
051768	x	c.2440C>T	p.Q814X	*De novo*	
062375	s	c.2443-1delG	IVS6-1delG		
060118	s	c.2498+2dupT	IVS7+2dupT	*De novo*	
062315	f	**c.2504_2508delATCTT**	**p.Y835fsX848**		3
800824	f	**c.2504_2508delATCTT**	**p.Y835fsX848**		3
076679	f	**c.2509_2512delCATT**	**p.H837fsX842**		
802426	f	**c.2509_2512delCATT**	**p.H837fsX842**		
061328	x	**c.2572C>T**	**p.R858X**		*De novo* in 3

(*continued*)

Table 2. (Continued)

Accession no.	*Type*	*DNA mutation*[a]	*Protein mutation*	*Parental testing*	*Previously reported*
074555	x	**c.2572C>T**	**p.R858X**		*De novo* in 3
801724	x	**c.2572C>T**	**p.R858X**		*De novo* in 3
806103	x	**c.2572C>T**	**p.R858X**		*De novo* in 3
070890	m/s	c.2613G>T	p.E871D[b]	*De novo*	
062987	s	c.2697+2T>G	IVS9+2T>G	One parent excluded	
071276	f	c.2737_2738insTC	p.Y913fsX925		
800699	x	c.2753G>A	p.W918X	*De novo*	
070907	x	c.2839C>T	p.R947X		2, *de novo* in 11
801407	x	c.2858G>A	p.W953X		
065597	f	c.2905_2906delAG	p.R969fsX993		
052425	s	c.2957+2T>G	IVS11+2T>G		
060339	x	**c.2959C>T**	**p.R987X**		3, *de novo* in 5
070946	x	**c.2959C>T**	**p.R987X**		3, *de novo* in 5
800117	x	**c.2959C>T**	**p.R987X**		3, *de novo* in 5
806836	x	**c.2959C>T**	**p.R987X**		3, *de novo* in 5
810347	x	**c.2959C>T**	**p.R987X**		3, *de novo* in 5
073135	m	c.3005C>T	p.Q1002R[b]	*De novo*	
064779	m	**c.3082A>G**	**p.I1028V**[b]		*De novo* in 1, 3, and 8
077115	m	**c.3082A>G**	**p.I1028V**[b]	*De novo*	*De novo* in 1, 3, and 8
064695	x	**c.3106C>T**	**p.R1036X**		2, 3
071885	x	**c.3106C>T**	**p.R1036X**		2, 3
075224	x	**c.3106C>T**	**p.R1036X**		2, 3
804863	x	**c.3106C>T**	**p.R1036X**		2, 3
903061	x	**c.3106C>T**	**p.R1036X**		2, 3
811229	f	c.3122dupT	p.L1041fsX1052		
061125	x	c.3205C>T	p.R1069X		12, *de novo* in 4 and 8
076312	f	c.3572_3573delAA	p.K1191fsX1206		
054292	f	c.3617_3619delTTGinsAATA	p.I1206fsX1207		
902200	x	**c.3646A>T**	**p.K1216X**		
903124	x	**c.3646A>T**	**p.K1216X**		
800035	x	c.3655C>T	p.R1219X		3, 9
805981	f	c.3693delA	p.K1231fsX1242		
054177	f	c.3728dupA	p.N1243fsX1262		
064752	x	c.3768C>G	p.Y1256X	*De novo*	
065763	s	c.3779-2A>G	IVS15-2A>G		3
071881	x	c.3802G>T	p.E1268X		
053821	f	c.3806_3811delTTAAAGinsA	p.F1269fsX1269		
053162	m	c.3881T>C	p.L1294P[b]		*De novo* in 4
807908	s	c.3989+1G>A	IVS16+1G>A		
061708	s	c.3990-2A>G	IVS16-2A>G		
063439	f	c.4012_4013delGG	p.G1338fsX1355		
077569	x	**c.4015C>T**	**p.R1339X**		2, 3, 11, *de novo* in 4
808924	x	**c.4015C>T**	**p.R1339X**		2, 3, 11, *de novo* in 4
052094	f	c.4138dupA	p.T1380fsX1385		8
078276	x	c.4164G>A	p.W1388X	*De novo*	
052779	f	c.4183delC	p.Q1395fsX1403		
061322	m/s	c.4185G>C	p.Q1395H[b]	*De novo*	*De novo* in 8
073529	s	c.4186-1G>A	IVS17-1G>A		
051749	f	c.4203_4204delTA	p.H1401fsX1420		
053271	x	c.4318C>T	p.Q1440X		
063442	x	**c.4393C>T**	**p.R1465X**	*De novo*	4
074759	x	**c.4393C>T**	**p.R1465X**		4
806837	x	**c.4393C>T**	**p.R1465X**		4
060639	x	c.4441A>T	p.K1481X		
074907	x	c.4480C>T	p.R1494X		2, 13
802505	s	c.4533+1G>A	IVS19+1G>A		
801879	x	c.4593G>A	p.W1531X		
062503	x	c.4601G>A	p.W1534X		12, *de novo* in 4
052966	f	c.4634delT	p.L1545fsX1545		
903559	x	c.4753G>T	p.E1585X		
062946	x	c.4795C>T	p.Q1599X		Inherited in 8

(*continued*)

Table 2. (Continued)

Accession no.	*Type*	*DNA mutation*[a]	*Protein mutation*	*Parental testing*	*Previously reported*
061334	x	c.4853G>A	p.W1618X		
077838	x	**c.5029C>T**	**p.R1677X**	*De novo*	
061465	x	**c.5029C>T**	**p.R1677X**		
065687	s	c.5050+1G>A	IVS22+1G>A		
806781	f	c.5054delT	p.L1685fsX1698		
806158	f	c.5086_5093delAAGAAGGT	p.K1696fsX1733		
053905	f	c.5094dupG	p.K1699fsX1736		
807855	x	c.5101C>T	p.Q1701X		
064486	x	c.5122C>T	p.Q1708X		
063670	f	c.5138_5141delTGGC	p.L1713fsX1730		
053226	f	c.5138_5141delTGGC c.5147_5148insGCCAGCTG	p.L1713fsX1737	*De novo*	
807173	f	c.5178_5179dupCT	p.Y1727fsX1732		
076269	m	c.5216T>G	p.L1739R[b]	*De novo*	
053286	x	c.5245A>T	p.R1749X		
063510	f	c.5250delA	p.Q1750fsX1752		
054190	x	**c.5428C>T**	**p.R1810X**		*De novo* in 4 and 11
902215	x	**c.5428C>T**	**p.R1810X**		*De novo* in 4 and 11
060225	s	c.5534+1G>A	IVS26+1G>A		*De novo* in 3 and 4
902057	f	c.5574delA	p.K1858fsX1868		
064246	f	c.5588delC	p.P1863fsX1868		
807509	s	c.5666-2A>C	IVS28-2A>C		
070476	f	c.5776delA	p.R1926fsX1929		
807429	x	c.5782C>T	p.Q1928X		
052662	x	c.5791C>T	p.Q1931X	*De novo*	
052314	x	**c.5833C>T**	**p.R1945X**	One parent excluded	4, *de novo* in 3
054428	x	**c.5833C>T**	**p.R1945X**		4, *de novo* in 3
800825	f	c.5960_5963delCTGT	p.P1987fsX2041		
062684	f	c.6018delA	p.K2006fsX2042		
804799	x	**c.6070C>T**	**p.R2024X**		1, 3, *de novo* in 4
802868	x	**c.6070C>T**	**p.R2024X**		1, 3, *de novo* in 4
064618	x	c.6079C>T	p.R2027X		*De novo* in 3
060386	s	c.6103+8T>C	IVS30+8T>C		*De novo* in 4
808893	x	c.6157C>T	p.R2053X		3, 5, *de novo* in 4
806949	x	c.6272G>A	p.W2091X		
062558	x	**c.6292C>T**	**p.R2098X**		10
800374	x	**c.6292C>T**	**p.R2098X**		10
062120	f	c.6320_6321delAC	p.H2107fsX2118		
053898	m	**c.6347T>A**	**p.I2116N**[b]	One parent excluded	
801826	m	**c.6347T>A**	**p.I2116N**[b]	*De novo*	
075271	x	c.6397C>T	p.Q2133X		
064459	f	c.6461delC	p.P2154fsX2214		
902770	f	c.6502delC	p.L2168fsX2214		
061870	f	c.6587_6589delCCGinsTA	p.T2196fsX2214		
053153	f	c.6746delA	p.D2249fsX2276	*De novo*	
803104	x	c.6757G>T	p.E2253X		
042727	s	c.6775+1G>A	IVS31+1G>A		
062825	x	**c.6850C>T**	**p.R2284X**		*De novo* in 6 and 8
077988	x	**c.6850C>T**	**p.R2284X**		*De novo* in 6 and 8
805191	x	**c.6850C>T**	**p.R2284X**		*De novo* in 6 and 8
074324	f	c.7027delC	p.Q2343fsX2442		
05865	x	c.7132G>T	p.E2378X	*De novo*	
052445	x	c.7195C>T	p.Q2399X		
061695	f	c.7249delA	p.R2417fsX2442		
061278	x	**c.7252C>T**	**p.R2418X**	*De novo*	3
902420	x	**c.7252C>T**	**p.R2418X**		3
076853	x	**c.7282C>T**	**p.R2428X**		3
809472	x	**c.7282C>T**	**p.R2428X**		3
063341	f	c.7328delA	p.D2443fsX2502		
075614	x	c.7367C>G	p.S2456X		13
060501	f	c.7418_7427del10	p.P2473fsX2499		

(*continued*)

Table 2. (Continued)

Accession no.	*Type*	*DNA mutation*[a]	*Protein mutation*	*Parental testing*	*Previously reported*
062920	x	c.7447G>T	p.E2483X		
062147	x	c.7636G>T	p.E2546X	Father mosaic	13
051996	f	c.7782delG	p.W2594fsX2595	*De novo*	
076047	f	c.7875_7876delGA	p.Q2625fsX2628		
062087	x	**c.7879C>T**	**p.R2627X**		3
064661	x	**c.7879C>T**	**p.R2627X**	One parent excluded	3
054199	x	**c.7891C>T**	**p.R2631X**		
060051	x	**c.7891C>T**	**p.R2631X**		
070279	x	**c.7891C>T**	**p.R2631X**		
800072	x	**c.7891C>T**	**p.R2631X**		
811668	x	**c.7891C>T**	**p.R2631X**		
065478	f	c.7919_7926delCTTTGACA	p.T2640fsX2649		
806062	f	c.7921_7922delTT	p.L2641fsX2651		
065062	x	c.8054G>A	p.W2685X	*De novo*	
061050	f	c.8078delG	p.G2693fsX2708	*De novo*	
077711	f	c.8452_8459dupAACCCTCT	p.L2820fsX2891		
062355	f	c.8565delA	p.K2855fsX2888		
05885	f	c.8962dupG	p.D2988fsX2989	*De novo*	*De novo* in 8
077415	del	delEX2_38	Whole gene deletion		1, 11
063780	del	delEX2	Exon 2 dele		
807132	dup	dupEX3	Exon 3 dup		

[a]Mutated bases in the human *CHD7* cDNA were numbered based on accession number NM_017780. Gene and protein nomenclature follows recommendations (den Dunnen and Antonarakis, 2001). Bold font indicates recurrent mutations.
[b]Conserved in zebrafish CHD7 protein.
[1]Vissers *et al.* (2004).
[2]Aramaki *et al.* (2006).
[3]Jongmans *et al.* (2006).
[4]Lalani *et al.* (2006).
[5]Sanlaville *et al.* (2006).
[6]Felix *et al.* (2006).
[7]Delahaye *et al.* (2007).
[8]Vuorela *et al.* (2007).
[9]Writzl *et al.* (2007).
[10]Gennery *et al.* (2008).
[11]Wincent *et al.* (2008).
[12]Lee *et al.* (2009).
[13]Asakura *et al.* (2008); Fujita *et al.* (2009).
x = premature stop codon; f = frameshift mutation; s = splice site mutation; m = missense mutation; del = deletion; dup = duplication; ins = insertion.

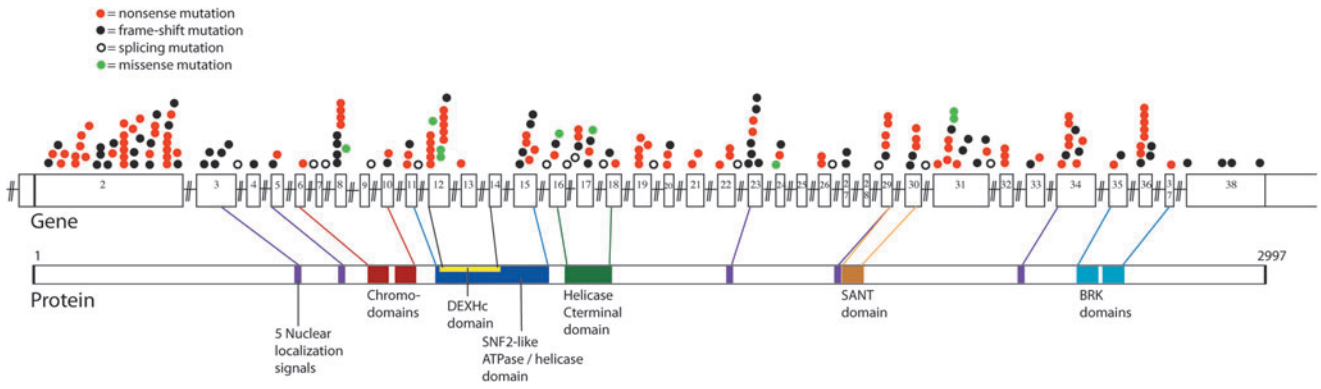

FIG. 1. The *CHD7* gene (top) and protein (bottom). Colored circles above exons 2–38 depict the location of 200 CHARGE-causing mutations. Overlapping circles indicate identical mutations. Protein domains are labeled and lines indicate where each protein domain is encoded on the gene. All DNA mutations that introduced stop codons or frameshifts were considered disease causing, as were mutations of the canonical splice donor–acceptor pair (GT-AG). Missense changes or other putative splicing changes were not considered disease causing unless the change was a *de novo* mutation not found in either parent or if it was reported in the literature as a *de novo* mutation.

Table 3. Sequence Variations with Unknown Significance

a. Rare missense variations, residue insertion/deletions, and variations that are silent coding changes or intron changes close to exon/intron junctions and have the potential to be splicing mutations

Accession no.	*DNA mutation*	*Protein variation*	*Previously published*	*Situation*	*Parent*	*Conservation*[a]
902591	c.123G>A	p.M41I				Yes
076175	c.257C>G	p.P86R			Identified in 1 parent	No (Q)
62883	c.561G>A	p.Q187Q				Similar (H, cac)
053498	c.712G>A	p.V238M		With G744S and A2160T		No (P)
53964	c.1029C>T	p.S343S				Similar (N)
074161	c.1122_1133dup12	p.N377_T378ins PNEH				Yes
5828	c.1672C>G	p.P558A		7 bp from junction		Yes
811103	c.1677G>A	p.S559S			Identified in 1 parent	No (P)
053117	c.2096G>C	p.S699T		1 bp from junction, putative splice		Yes
806511	c.2097-5delT	IVS3-5delT		5 bp from junction		No
800842	c.2182G>A	p.D728N				Similar (E)
65571	c.2196A>G	p.P732P				Yes
62573	c.2498+6T>G	IVS7+6T>G		6 bp from junction		Yes
072511	c.2499-3C>G	IVS7-3C>G		3 bp from junction		Yes
065403	c.2680A>G	p.T894A		In chromodomain	Identified in 1 parent	Yes
061052	c.2720A>C	p.K907T		In chromodomain		Yes
063067	c.2750C>T	p.T917M		In chromodomain	Identified in 1 parent	Yes
903397	c.2813G>A	p.R938K				Yes
072071	c.2831G>A	p.R944H		5 bp from junction	Identified in 1 parent	Yes
053964	c.2840G>A	p.R947Q		5 bp from junction	Identified in 1 parent	Yes
71691	**c.3202-5T>C**	**IVS12-5T>C**		5 bp from junction		Yes
802765	**c.3202-5T>C**	**IVS12-5T>C**		5 bp from junction		Yes
801050	**c.3378+5G>T**	**IVS13+5G>T**		5 bp from junction		Yes
801088	**c.3378+5G>T**	**IVS13+5G>T**		5 bp from junction		Yes
051914	c.3607G>C	p.E1203Q		In SNF2-N domain		Yes
903485	c.3623T>A	p.V1208D		In SNF2-N domain		Yes
802422	c.3942G>A	p.Q1314Q				Yes
072739	c.3965T>C	p.L1322P		In HeLICc domain		Yes
65403	c.3989C>G	p.R1330R		2 bp from junction, putative splice		Yes
065012	c.4033C>T	p.R1345C		In HeLICc domain	Identified in 1 parent	Yes
807330	c.4247C>G	p.T1416R				Yes
811007	c.4369A>C	p.K1457Q		With V2931M	Identified in 1 parent	Yes
072994	c.4727T>G	p.F1576C				Similar (Y)
800514	c.4849G>A	p.G1617S		2 bp from junction, putative splice		Yes
060081	c.5050G>A	p.G1684S	13	1 bp from junction, putative splice		Yes
063000	c.5373C>A	p.D1791E			identified in 1 parent	Yes
070154	**c.5405-7G>A**	**IVS25-7G>A**	2, 3	7 bp from junction, putative splice		No
072674	**c.5405-7G>A**	**IVS25-7G>A**	2, 3	7 bp from junction, putative splice		No
075645	**c.5405-7G>A**	**IVS25-7G>A**	2, 3	7 bp from junction, putative splice		No
808268	**c.5405-7G>A**	**IVS25-7G>A**	2, 3	7 bp from junction, putative splice		No

(*continued*)

TABLE 3. (CONTINUED)

Accession no.	DNA mutation	Protein variation	Previously published	Situation	Parent	Conservation[a]
810826	**c.5405-7G>A**	**IVS25-7G>A**	2, 3	7 bp from junction, putative splice		No
074838	**c.5597A>G**	**p.D1866G**		11 bp from junction		Yes
053177	**c.5597A>G**	**p.D1866G**		11 bp from junction		Yes
64239	c.5841A>G	p.E1947E				Similar (D)
053492	c.5848G>A	p.A1950T			Identified in 1 parent	Yes
064827	c.5894+5G>A	IVS29+5G>A		5 bp from junction		Yes
805662	c.5905_5907del AGA	p.R1969del		11 bp from junction	Identified in 1 parent	Yes
054355	c.6103+5G>T	IVS30+5G>T		5 bp from junction		No
806941	**c.6194G>A**	**p.R2065H**				Yes
903222	**c.6194G>A**	**p.R2065H**			Identified in 1 parent	Yes
811609	c.6250A>G	p.S2084G				Yes
810303	**c.6308G>A**	**p.G2103D**				Yes
064850	**c.6308G>A**	**p.G2103D**				Yes
052986	c.6339T>C	p.D2113D				Yes
051356	c.6363G>A	p.E2121E				Yes
804307	c.6673G>A	p.A2225T				No (L)
62708	c.6936G>A	p.K2312K		1 bp from junction, putative splice		Yes
805742	c.6955C>T	p.R2319C	3, 6, 14		Identified in parent and sibling	Yes
077040	c.6989G>C	p.G2330A				Yes
61994	c.7158G>A	p.L2386L		7 bp from junction		Similar (V)
054238	**c.7165-4A>G**	**IVS33-4A>G**	3	4 bp from junction		No
051771	**c.7165-4A>G**	**IVS33-4A>G**		4 bp from junction		No
05828	**c.7165-4A>G**	**IVS33-4A>G**		4 bp from junction		No
054005	c.7485G>T	p.R2495S			Identified in 1 parent	No (−)
051390	c.7578C>T	p.S2528S				No (G)
071896	c.8047C>T	p.P2683S		In BRK domain	Identified in 1 parent	Yes
070710	c.8104C>T	p.R2702C			Identified in 1 parent	Yes
901480	c.8197G>A	p.A2733T				Yes
052878	c.8569T>G	p.S2857A				Yes
800917	c.8874C>T	p.A2958A				Similar (S)
811007	c.8791G>A	p.V2931M		With K1457Q	Identified in 1 parent	Yes
808890	c.8879_8881dup AGA	p.E2960_S2961insK			Identified in 1 parent	Yes

b. 32 sequence variations identified as polymorphisms

Accession no.	DNA mutation	Protein variation	Previously published	Situation	Parental testing	Zebrafish conservation[a]	n	SNP ID no.
62599	c.216T>C	p.Y72Y				Similar (F)	5	rs16926453
070640	c.307T>A	p.S103T	11 (4/180 controls)			Yes	6	rs41272435
53498	c.309G>A	p.S103S				Yes	3	
05913	c.602A>G	p.Q201R			Identified in 1 parent	Yes	4	
53498	c.657C>T	p.G219G				No (−)	5	
053103	c.1018A>G	p.M340V	8		1	No (S)	6	rs41305525
803481	c.1105C>G	p.P369A			In parent and 2 relatives	No (−)	1	

(*continued*)

Table 3. (Continued)

Accession no.	*DNA mutation*	*Protein variation*	*Previously published*	*Situation*	*Parental testing*	*Zebrafish conservation*[a]	n	*SNP ID no.*
05885	c.1397C>T	p.S466L	6		1	No (P)	1	rs71640285
053015	c.1536A>G	p.P512P				Yes	3	
902192	c.1565G>T	p.G522V	6	Homozygous		No (A)	1	
62147	c.1907G>T	p.G636V		With E2546X		No (I)	1	
078278	c.2053_58 dupGAAAA	p.A685_K686dup	11 (3/80 controls)		Identified in 1 parent	Yes	6+	
53498	c.2124T>C	p.S708S				No (G)	6	
053498	c.2230G>A	p.G744S	8, 11	1 with V238M and A2160T		Yes	3	
77306	c.2361C>A	p.S787S				No (−)	3	
060685	c.3379-33A>G	IVS13-33A>G		33 bp from junction		No	1	rs45461501
60036	c.5051-4C>T	IVS22-4C>T				No	6+	rs71640288
51581	c.5307C>T	p.A1769A		7 bp from junction		No (L)	6+	rs16926499
060386	c.6103+8T>C	IVS30+8T>C	4			Yes	6+	rs3763592
52986	c.6111C>T	p.P2037P		8 bp from junction		Yes	3	rs41312170
062383	c.6135G>A	p.P2045P				Yes	6+	rs6999971
052668	c.6276G>A	p.E2092E	4			Yes	6+	rs2068096
60685	c.6282A>G	p.G2094G				Yes	6	rs41312172
053498	c.6478G>A	p.A2160T	8, 11	1 with V238M and G744S		No (−)	2	rs61753399
71954	c.6738G>A	p.E2246E	8, 11			Similar (D)	3	rs61729627
804229	c.6833T>C	p.A2274A				Yes	1	rs61743849
053179	c.7356A>G	p.T2452T	4			Similar (S)	6+	rs2272727
061251	c.7579A>C	p.M2527L			Identified in 1 parent	Yes	5	
53498	c.7590A>G	p.K2530K				Yes	3	rs61742801
53162	c.8355C>T	p.A2785A		With L1294P		No (G)	1	
070556	c.8416C>G	p.L2806V	8, 11		Identified in 1 parent	Yes	2	rs45521933
061484	c.8950C>T	p.L2984F			Identified in 1 parent	Similar (M)	3	

The inherited/*de novo* status of most is unknown unless otherwise indicated.

[a]Conservation with zebrafish CHD7 protein is noted for coding mutations. Conservation with zebrafish CHD7 DNA sequence is noted for possible splice variations.

[1]Vissers *et al.* (2004).
[2]Aramaki *et al.* (2006).
[3]Jongmans *et al.* (2006).
[4]Lalani *et al.* (2006).
[5]Sanlaville *et al.* (2006).
[6]Felix *et al.* (2006).
[7]Delahaye *et al.* (2007).
[8]Vuorela *et al.* (2007).
[9]Writzl *et al.* (2007).
[10]Gennery *et al.* (2008).
[11]Wincent *et al.* (2008).
[12]Lee *et al.* (2009).
[13]Asakura *et al.* (2008); Fujita *et al.* (2009).
[14]Holak *et al.* (2008).

Blue-shaded rows: five mutations are 1–2 bp from an intron and we believe these to be splicing mutations. The IVS25-7G>A mutation, in five additional samples, has been previously reported but has not yet been identified as a *de novo* mutation.

Blues boxes: algorithm-predicted splice site mutations.
Green boxes: missense mutations.
Gray boxes: deletion, duplication, insertion mutations.
Bold font indicates recurrent mutations.

Such results are reported as being variants of unknown significance in patient reports. In another 18 cases, where we were able to test one or both parents, the variant was observed in a parent. However, without further information, these missense changes are difficult to classify. Germline transmission has been reported and may be due to germline mosaicism, somatic mosaicism, or inheritance of the mutation from a mildly affected parent. When inheritance of a novel missense change is observed in a molecular diagnostic setting, it is difficult to know if the parent is mosaic for a pathogenic

mutation or if the variant is a benign polymorphism (Zlotogora, 1998). In these cases, clinical evaluation of the parent is recommended to the referring physician.

Twenty-two variants that may affect splicing were identified based on prediction algorithms such as SIFT (Lowe, 2004) and PolyPhen (Ramensky *et al.*, 2002). Two of these were synonymous changes that could affect splicing, as has been observed in other diseases (Eriksson *et al.*, 2003). Table 3a includes nonsynonymous missense variants that are presumed to be very rare, as they have been observed only once or twice in the 1284 alleles we have tested and were not found in the SNP databases. Without further information, we cannot determine the pathogenicity of these variants.

Table 3b lists variants characterized as benign polymorphisms. Twenty-nine polymorphisms were found in multiple individuals in this report or in previous reports, two were found in individuals also carrying disease-causing mutations, and one was homozygous.

A total of 370 specimens had no detectible mutations by sequencing, and we were able to test only 23 specimens for exonic copy number changes. It is not unusual to observe several polymorphisms in the *CHD7* gene, and the observation of heterozygous positions ensures the presence of both alleles. In our negative samples, 76 altogether lacked heterozygous polymorphisms and another 88 specimens had only one to two polymorphisms. Although copy number changes are not common in the *CHD7* gene, these samples (26% of all samples submitted) may be good candidates for deletion and duplication testing using other methods for detection.

Discussion

This report serves as a summary of the findings of *CHD7* mutation analysis observed by one clinical diagnostic laboratory. Unlike other publications, we have not performed clinical evaluations on the patients in whom the analyses were performed, and these data must be regarded with that in mind.

We detected *CHD7* mutations in 203 of 642 ($\sim$32%) patient specimens referred to GeneDx for clinical testing. One hundred twenty of the 203 *CHD7* mutations have not been previously reported. Consistent with previous reports, most of the mutations we detected are nonsense ($n = 107$; 52.7%) and frameshift ($n = 69$; 34%) mutations and are predicted to cause loss of function. Splicing ($n = 15$; 7.4%), missense ($n = 9$; 4.4%), and copy number changes ($n = 3$; 1.5%) are less common. There is a higher percentage of stop codons in our cohort than in the published literature (52.7% vs. 35.4%). Of 189 published mutations, there were 35.4% stop mutations, 33.3% frameshifts, 7%–15% splicing mutations, 6%–13% missense mutations, and 3% large deletion/duplications (Vissers *et al.*, 2004; Felix *et al.*, 2006; Jongmans *et al.*, 2006; Lalani *et al.*, 2006; Sanlaville *et al.*, 2006; Aramaki *et al.*, 2007; Vuorela *et al.*, 2007; Asakura *et al.*, 2008; Bergman *et al.*, 2008; Gennery *et al.*, 2008; Wincent *et al.*, 2008).

Variants found in 11% ($n = 72$) of our 642 patient samples could not be classified as either pathogenic or benign, which clearly underscores the need for a functional assay, or at least the availability of parental samples for follow-up. The observation of mutations in 32% of the specimens evaluated is far lower than the 58%–64% reported by other groups. This reflects the variability in clinicians' use of molecular diagnostic testing, including the fact that many clinicians are considering a number of diagnoses in the differential when faced with a child who has some findings indicative of CHARGE syndrome.

Notably, there are 25 different mutations that have been observed more than once in our cohort. Six of these recurrent mutations were observed in four or more patient specimens. Each of these is a nonsense change involving a CGA arginine codon (R312X, R494X, R858X, R987X, R1036X, R2631X). This is consistent with reports that the CG dinucleotide is hypermutable to TG (Youssoufian *et al.*, 1988; Antonarakis *et al.*, 2000), making the arginine CGA codon uniquely vulnerable to transition to a nonsense mutation. Human CHD7 contains 27 arginine CGA codons. Despite these mutation hot spots, this should not influence how *CHD7* mutation analysis is performed, given that overall these account for only a fraction (14.2%) of the total observed mutations.

In our study, we tested the DNA samples of both parents for the presence of a variant identified in their child in 25 families. This included testing for 12 nonsense, 6 frameshift, 6 missense, and 1 splice site mutation. Of these, only one mutation was identified in a parent and all other mutations had arisen *de novo*. Mosaicism for a p.E2546X nonsense mutation was observed in this individual's specimen. Sixteen cases of germline transmission of *CHD7* mutation have been reported (Jongmans *et al.*, 2006; Lalani *et al.*, 2006; Delahaye *et al.*, 2007; Jongmans *et al.*, 2008; Vuorela *et al.*, 2008; Wincent *et al.*, 2008; Pauli *et al.*, 2009). Some of these cases involve an affected or mildly affected parent, whereas in other cases the carrier parent is reported as unaffected. Two affected siblings have been reported in a family where the father had no detectable *CHD7* gene mutation in lymphocyte DNA, but showed a mutation in ¼ of his sperm (Pauli *et al.*, 2009).

The *CHD7* gene is large at over 188 kb. Most laboratories seek mutations in the $\sim$9 kb that constitute the protein coding sequence and intron–exon junctions. As with most diagnostic tests, mutation analysis does not include the promoter, noncoding exons, or introns. Future full-gene sequencing using "next-generation" methods may increase the clinical sensitivity of diagnostic testing of CHARGE syndrome, revealing mutation deep in introns and promoter regions in other patients who carry a clinical diagnosis but are mutation negative using current methods.

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C.S. is currently an employee of Medco Health Solutions, Inc. Her contributions to this document are not to be construed as reflecting the views of Medco Health Solutions, Inc.

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