Identification of 217 unreported mutations in the *F8* gene in a group of 1,410 unselected Italian patients with hemophilia A

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Abstract To provide a National database, 1,410 unrelated hemophilia A (HA) patients were investigated using screening methods [denaturing high-performance liquid chromatography (DHPLC), conformational-sensitive gel electrophoresis (CSGE)] and/or direct sequencing. *F8* gene mutations were identified in 877 (81%), 146 (82%), and 133 (89%) families with severe, moderate, or mild HA, respectively. Among the 382 different mutations detected, 217 (57%) have not previously been reported in the F8 Haemophilia A Mutation, Structure, Test and Resource Site (HAMSTeRS) database. Mutations leading to a null allele accounted for 82, 15%, and less than 1% of severe, moderate, or mild HA, respectively. A missense mutation was identified in 16%, 68%, and 81% of severe, moderate,

or mild HA, respectively. They included 105 missense mutations (48%), 41 small deletions (19%), 25 splice site mutations (12%), 24 nonsense mutations (11%), 18 insertions (8%), three large deletions (1%), and one deletion plus insertion. Unreported mutations were distributed throughout the *F8* gene, as they affected all *F8* exons but exon 20. We report a wide spectrum of mutations collected in a large National database. The type of mutation was a strong predictor of the clinical phenotype. This database is expected to considerably improve the genetic counseling and medical care of HA families in Italy.

Keywords Hemophilia A · *F8* gene · Mutations · Phenotype

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Introduction

Hemophilia A (HA) is an X-linked bleeding disorder caused by heterogeneous mutations in the *Factor 8* (*F8*) gene. Its incidence is estimated at 1:5,000–10,000 in men (Kazazian et al. 1995). The *F8* gene maps to the distal end of the long arm of X-chromosome (Xq28) and spans 186 kb of genomic DNA. It consists of 26 exons and encodes a mature protein of 2,332 amino acids (Kazazian et al. 1995). Over the last decades, rapidly increasing numbers of causative gene alterations have been described in different ethnic groups. Actually, more than 900 mutations within the *F8* coding and untranslated regions have been identified and listed in the F8 Haemophilia A Mutation, Structure, Test and Resource Site (HAMSTeRS) mutation database (URL: <http://europium.csc.mrc.ac.uk>) (Gitschier et al. 1984). These findings further support the concept that HA has a very high mutational heterogeneity and represents a challenge for the provision of genetic services because carrier and prenatal diagnosis cannot be based on the screening of a limited number of common mutations (Kemball-Cook et al. 1998). The exponential discovery rate of new genomic alterations, leading to HA, as well as the need for comparative studies of different population's mutation frequencies, requires recording their population-wide spectrum in mutation databases. These databases are likely to facilitate genetic consultation of patients' families and can help optimize national DNA diagnostic services also by enhancing awareness among clinicians, patients, and the general public. Finally, mutation analysis of a given population is useful for further understanding structural and functional aspects of the mutant protein and the correlation between genotype and phenotype. Therefore, the Italian Association of Hemophilia Centres (AICE) planned to set up a National database aiming at characterizing the *F8* gene mutations to elucidate molecular basis of HA in Italy and to provide insight into protein structure-function relationships, unraveling the correlation (if any) between genotype and clinical phenotype.

In this study, we report phenotypic and genotypic data from 1,410 independent HA families comprising about 82% of Italian HA patients. These data strengthen the relevance for a National Hemophilia Registry that is expected to considerably improve the medical care of HA families in Italy with respect to treatment and genetic counseling.

Materials and methods

The study was carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained

from all participants. All patients involved in this study are regularly followed at one of 49 hemophilia treatment centers (see "Appendix") spread all over the country and belonging to AICE (URL: <http://www.aiceonline.it>), an organization founded with the aim of organizing national clinical and research activities in inherited bleeding disorders. Patients were considered to have HA according to the international consensus of the 2001 International Society on Thrombosis and Haemostasis (ISTH) Factor VIII and Factor IX Subcommittee (White et al. 2001). All known HA patients are registered and followed at one of these centers. For each patient, clinical and laboratory data (including FVIII clotting activity and inhibitor) are recorded.

DNA collection

A blood sample (5–10 mL) was collected in ethylenediaminetetraacetate (EDTA) or sodium citrate from each patient and stored at -20°C until sent to one of the nine laboratories that performed the genetic analysis. Isolation of DNA from leukocytes was carried out according to standard procedures (Miller et al. 1988).

PCR detection of the introns 1 and 22 inversion

Intron 1 inversions were detected by polymerase chain reaction (PCR), as reported earlier (Bagnall et al. 2002). Long PCR for detecting the intron 22 inversion was performed as previously described (Liu et al. 1998).

Amplification of the *F8* gene

PCR was carried out according to standard procedures (D'Andrea et al. 2002). A total of 14 kb of the *F8* gene, including the entire coding sequence, exon–intron junctions, and part of the 5' and 3' untranslated regions, were amplified by PCR. Most of the primers and amplification conditions have been previously reported; primer sequences, annealing temperatures, and the size of PCR fragments are available on request.

Mutation detection screening

Mutational analysis was performed through all of the coding exons and the exon/intron boundaries of the *F8* gene using denaturing high-performance liquid chromatography (DHPLC) analysis in four of the nine laboratories (Firenze, Genova, Parma, and Vicenza), whereas another

one (Castelfranco Veneto) used conformational-sensitive gel electrophoresis (CSGE). The success rate ranged from 80% to 95%. All amplification products that showed an abnormal pattern were then analyzed by direct sequencing. The remaining four laboratories (Foggia, Milano, Napoli, and Padova) did not employ any screening method, and samples were subjected to direct gene sequencing.

DNA sequencing

Amplified DNA fragments were purified and subjected to direct cycle sequence analysis using the Taq dye-deoxy terminator method and an ABI PRISM 3100 genetic Analyzer sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. All the nucleotide changes identified were confirmed by repeating the PCR and sequencing reactions. To address whether new mutations identified would be a polymorphism, 100 control subjects from the same ethnic background were investigated. Mutation nomenclature was according to the F8 HAMSTeRS mutation database (URL: <http://europium.csc.mrc.ac.uk>).

Splice-site prediction

Missense mutations and mutations at or near the splice junction consensus sequences were analyzed by the Splice Site Prediction program (http://www.fruitfly.org/seq_tools/splice.html) to predict the changes in RNA splicing.

Results

F8 gene mutations were identified in 1,156 out of 1,410 unrelated families with a history of HA, representing a mutation detection rate of 82%. Among these unrelated families, at least one causal *F8* mutation was identified in 877 (81%), 146 (82%), and 133 (89%) families with severe, moderate, or mild HA, respectively. Among severe HA patients, in addition to intron 22 and 1 inversion, 272 different mutations were recorded, including 100 missense mutations, 63 small deletions, 47 nonsense mutations, 25 splice-site mutations, 24 small insertions, 11 large deletions (seven spanning more than one domain), and two involving different mechanisms (deletion + insertion; missense/splicing) (Fig. 1a). In patients presenting with moderate HA, 83 different mutations were recorded, including 59 missense mutations, six splice-site mutations, six small deletions, six nonsense mutations, five small insertion, and one involving a different mechanism (duplication of exon 13) (Acquila et al. 2004) (Fig. 1b).

Fig. 1 Distribution of the different *F8* mutation types in unrelated hemophilia (HA) families according to the clinical phenotype. **a** Severe HA, **b** moderate HA, **c** mild HA

Finally, in mild HA patients, 57 different mutations were found, including 50 missense mutations, five splice-site mutations, one small deletion, and duplication of exon 13

(Acquila et al. 2004) (Fig. 1c). Overall, mutations that are likely to give rise to a null allele (inversions of an intron, deletions, insertions, and nonsense mutations) accounted for 82% of severe, 15% of moderate, and less than 1% of mild HA families. On the other hand, a missense mutation was identified in 16% of severe, 68% of moderate, and 81% of mild HA families. Mutations predicted to affect *F8* mRNA splicing occurred in 4% of severe, 16% of moderate, and 5% of mild HA families, suggesting that for most of splice-site mutations, by escaping aberrant splicing, a rescue of low levels of normal transcripts may take place.

Among the 384 different mutations detected, 217 (57%) have not previously been reported in the *F8* HAMSTeRS mutation database. They include 105 missense mutations (48%), 41 small deletions (19%), 25 splice-site mutations (12%), 24 nonsense mutations (11%), 18 insertions (8%), three large deletions (1%), and one deletion + insertion (Table 1). Unreported mutations were distributed throughout the *F8* gene, as they affected all *F8* exons but exon 20.

Exon 14 accounts for approximately 43% (3,106 out of 7,227 bp) of the coding region. Unreported mutations were found more frequently in exon 14, with 47 of 233 different mutations (21%) occurring in this portion of the *F8* gene. Novel mutations identified consisted mainly in premature terminal codon-causing mutations with 19 small deletions (41%), 11 insertions (23%), 11 nonsense mutations (23%), two splice-site mutations (4%), and only four missense mutations (9%).

Discussion

We report herein the largest series of *F8* analysis in HA patients issued in Italy. The vast majority of Italian families with HA, especially those with the severe cases, were included in this study, and it may be considered to be representative for the purpose of a population-based study of mutational heterogeneity. Actually, we were able to find 384 different mutations among 1,410 unrelated families. In accordance with previous data, more than 25% of these unrelated families of our cohort presented a novel mutation (Ljung and Sjörin 1999; Habart et al. 2003; Oldenburg and Pavlova 2006). This reflects the high degree of heterogeneity of the *F8*-affecting mutations outside inversion of introns 22 and 1. Our study confirms the well-known correlation between the type of mutation and the severity of HA. The type of mutations found was in agreement with results reported in other settings (Oldenburg and Pavlova 2006; Fernández-López et al. 2005; Jayandharan et al. 2005; Deszo et al. 2006). As would be expected, most severe HA patients carry a molecular defect leading to a null allele (large deletion,

inversion, nonsense, and insertion/deletion mutations), whereas missense mutations have been found in the majority of moderate (68%) and mild HA patients (81%). Likewise, most mutations predicted to affect *F8* mRNA splicing occurred in moderate or mild HA families, suggesting that low levels of normal transcripts may escape aberrant splicing. To improve genetic counseling and medical care of HA families, mutations identified in this work will be available at the HAMSTeRS mutation database.

Exon 14 represents about one half of the coding region and encodes for the *F8* B domain, a region lacking in procoagulant activity that is spliced out from the mature protein. This finding explains the occurrence of very few missense mutations in the central portion of exon 14 in our series and in other settings (see: HAMSTeRS mutation database).

In conclusion, we report a large National database in which a wide spectrum of new mutations has been collected. The type of mutation was a strong predictor of the clinical phenotype. This database will be of great value for genetic counseling in HA and is expected to considerably improve the medical care of HA families in Italy.

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Appendix

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Table 1 *F8* mutations identified unreported in the Haemophilia A Mutation, Structure, Test and Resource Site (HAMSTeRS) mutation database

Location	Nucleotide	Protein	Type	Effect	Clinical phenotype
Exon 1	c.-43 C > T		substitution	Transcription	Mild
Exon 1	c.-25 A > G		Substitution	Transcription	Severe
Exon 1	c.38 T > G	p.Leu-7Arg	Substitution	Missense	Severe
Exon 1	c.63 A > G	p.Arg3Gly	Substitution	Missense	Severe
Exon 1	c.72_188 del15bp	p.Tyr5_Val10del	Deletion	Small deletion	Moderate
Exon 1	c.102 C > G	p.Asp15Glu	Substitution	Missense	Mild
Exon 1	c.104 A > G	p.Tyr16Cys	Substitution	Missense	Severe
Intron 2	c.144 -3 C > G		Substitution	Splicing	Severe
Intron 2	c.144 -2 A > T		Substitution	Splicing	Severe
Exons 2–6			Deletion	Large deletion	Severe
Exon 2	c.202_203 delCT	p.Thr49del	Deletion	Small deletion	Severe
Exon 2	c.214 G > C	p.Glu53Asp	Substitution	Missense	Mild
Exon 3	c.275 G > A	p.Gly73Asp	Substitution	Missense	Severe
Exon 3	c.276 C > T	p.Pro74Ser	Substitution	Missense	Moderate
Exon 3	c.288_293 del GGCTGA	p.Gln77_Ala78del	Deletion	Small deletion	Severe
Exon 3	c.308 T > A	p.Val84Glu	Substitution	Missense	Severe
Exon 3	c.339 T > A	p.His94Gln	Substitution	Missense	Severe
Exon 3	c.341 C > T	p.Pro95Leu	Substitution	Missense	Moderate
Exon 3	c.343 T > G	p.Val96Gly	Substitution	Missense	Moderate
Exon 3	c.347_353 delGTCTTCA	p.Ser97_His99del	Deletion	Small deletion	Severe
Exon 3	c.350 T > C	p.Leu98Pro	Substitution	Missense	Severe
Exon 3	c.353 A > G	p.His99Arg	Substitution	Missense	Moderate
Exon 2	c.360 G > T	p.Gly102Val	Substitution	Missense	Moderate
Exon 3	c.362 G > C	p.Gly102Ala	Substitution	Missense	Severe
Exon 3	c.368 C > G	p. Ser104Stop	Substitution	Nonsense	Severe
Exon 3	c.392 A > G	p.Ile76Val	Substitution	Missense	Mild
Intron 3	c.388 + 5 G > A		Substitution	Splicing	Severe
Exon 4	c.403 G > T	p.Asp116Tyr	Substitution	Missense	Severe
Exon 4	c.427_428 delGA	p.Glu124del	Deletion	Small deletion	Severe
Exon 4	c.436 A > T	p.Lys127Stop	Substitution	Nonsense	Severe
Exon 3	c.440 G > A	p.Ala92Thr	Substitution	Missense	Severe
Exon 4	c.446 C > T	p.Pro130Leu	Substitution	Missense	Severe
Exon 4	c.450_51 insT	p.Gly132ins	Insertion	Insertion	Severe
Exon 4	c.484_85 delGA	p.Glu143del	Deletion	Small deletion	Severe
Exon 4	c.518 T > G	p.Leu154Arg	substitution	Missense	Mild
Exon 4	c.523 A > G	p.Tyr156Cys	Substitution	Missense	Moderate
Exon 4	c.555 G > A	p.Asp167Lys	Substitution	Missense	Moderate
Exon 4	c.575 T > C	p.Ile173Thr	Substitution	Missense	Moderate
Exon 4	c.578 G > A	p.Gly174Glu	Substitution	Missense	Severe
Exon 4	c.584 T > G	p.Leu176Arg	Substitution	Missense	Severe
Intron 4	c.602 -1 G > A		Substitution	Splicing	Severe
Intron 4	c.601 + 5 G > T		substitution	Splicing	Mild
Intron 4	c.602-13 + c.602_615del		Deletion	Small deletion	Severe
Exons 5–10			Deletion	Large deletion	Severe
Exon 5	c.648_49 ins10bp	p.Lys198ins	Insertion	Insertion	Severe
Exon 5	c.650 T > C	p.Lys198Pro	Substitution	Missense	Severe
Exon 5	c.654 delT	p.Phe199del	Deletion	Small deletion	Severe
Exon 5	c.668_670 delAAG	p.Glu204del	Deletion	Small deletion	Severe

Table 1 continued

Location	Nucleotide	Protein	Type	Effect	Clinical phenotype
Intron 5	c.670 + 6 A > G		Substitution	Splicing	Mild
Exon 6	c.677_9delGTT	p.Ser247_Arg248delinsArg	Deletion	Small deletion	Severe
Exon 6	c.686 C > G	p.Ser210Stop	Substitution	Nonsense	Severe
Exon 6	c.709 C > T	p.Gln218Stop	Substitution	Nonsense	Severe
Exon 6	c.741 G > A	p.Trp228Stop	Substitution	Nonsense	Severe
Exon 6	c.758 T > G	p.Val234Gly	Substitution	Missense	Severe
Exon 6	c.761 A > G	p.Asn235Ser	Substitution	Missense	Severe
Exon 6	c.763 G > T	p.Gly236Cys	Substitution	Missense	Severe
Exon 6	c.776 G > C	p.Arg240Thr	Substitution	Missense	Mild
Exon 6	c.785 delC	p.Pro243del	Deletion	Small deletion	Moderate
Intron 6	c.787 + 5 G > A		Substitution	Splicing	Severe
Exon 7	c.886 C > T	p.Leu277Phe	Substitution	Missense	Severe
Exon 7	c.919 delA	p.Ile288del	Deletion	Small deletion	Severe
Exon 7	c.967 G > A	p.Gly304Arg	Substitution	Missense	Severe
Exon 7	c.980 T > G	p.Leu308Arg	Substitution	Missense	Severe
Exon 7	c.986 G > A	p.Cys310Tyr	Substitution	Missense	Severe
Exon 8	c.1030 A > C	p.Lys325Glu	Substitution	Missense	Mild
Exon 8	c.1202 G > C	p.Trp382Cys	Substitution	Missense	Mild
Exon 9	c.1313 T > C	p.Ile419Tyr	Substitution	Missense	Mild
Exon 9	c.1315 G > T	p.Gly420Cys	Substitution	Missense	Severe
Exon 9	c.1316 G > C	p.Gly420Ala	Substitution	Missense	Moderate
Exon 9	c.1322 T > C	p.Tyr423Asn	Substitution	Missense	Severe
Exon 9	c.1364 T > C	p.Phe436Ser	Substitution	Missense	Severe
Exon 9	c.1396 G > A	p.Gly447Arg	Substitution	Missense	Severe/moderate
Exon 9	c.1414 T > A	p.Leu453His	Substitution	Missense	Moderate
Intron 9	c.1443 + 1 G > C		Substitution	Splicing	Moderate
Intron 9	c.1443 + 1 G > T		Substitution	Splicing	Severe
Exon 10	c.1464_1465 insCCAGAC	p.Ser470ins	Insertion	Insertion	Moderate
Exon 10	c.1473_1474 insCCAGAC	p.Tyr473ins	Insertion	Insertion	Moderate
Intron 10	c.1538–18 G > A		Substitution	Splicing	Moderate
Intron 10	c.1538–1 A > G		Substitution	Splicing	Severe
Intron 10	c.1537 + 1G > C		Substitution	Splicing	Severe
Exon 11	c.1582_88 delTTCAAAT	p.Phe509_Tyr511del	Deletion	Small deletion	Severe
Exon 11	c.1640 G > A	p.Cys528Tyr	Substitution	Missense	Moderate
Exon 11	c.1738 C > T	p.Gln561Stop	Substitution	Nonsense	Severe
Exon 11	c.1748 delA	p.Asn564del	Deletion	Small deletion	Severe
Exon 12	c.1810 T > C	p.Trp585Arg	Substitution	Missense	Severe
Exon 12	c.1811 G > A	p.Trp585Stop	Substitution	Nonsense	Severe
Exon 12	c.1813 T>C	p.Tyr586His	Substitution	Missense	Severe
Exon 12	c.1814 A > G	p.Tyr586Cys	Substitution	Missense	Severe
Exon 12	c.1845 delC	p.Pro596del	Deletion	Small deletion	Severe
Exon 12	c.1874_75 delCA	p.Pro606 del	Deletion	Small deletion	Severe
Exon 12	c.1897 A > G	p.Met614Val	Substitution	Missense	Mild
Intron 12	c.1904–2A > G		Substitution	Splicing	Severe
Intron 12	c.1904–1G > A		Substitution	Splicing	Severe
Intron 12	c.1903 + 2 T > G		Substitution	Splicing	Severe
Exon 12	c.1909 G > T	p. Glu581Asp	Substitution	Missense	Severe

Table 1 continued

Location	Nucleotide	Protein	Type	Effect	Clinical phenotype
Exon 13	c.1913 G > A	p.Gly619Asp	Substitution	Missense	Severe
Exon 13	c.1945 T > C	p.Cys630Arg	Substitution	Missense	Severe
Exon 13	c.1966 T > C	p.Trp637Arg	Substitution	Missense	Severe
Exon 13	c.1966 T > G	p.Trp637Gly	Substitution	Missense	Moderate
Exon 13	c.1991 A > G	p.Gln645Arg	Substitution	Missense	Moderate
Exon 13	c.2029 T > G	p.Phe658Val	Substitution	Missense	Moderate
Exon 13	c.2058_2059 delAC	p.Thr668del	Deletion	Small deletion	Severe
Exon 13	c.2061 C > T	p.Pro672Ser	Substitution	Missense	Severe
Exon 13	c.2078 C > G	p.Ser674Stop	Substitution	Nonsense	Severe
Exon 13	c.2081 G > A	p.Gly675Glu	Substitution	Missense	Severe/moderate
Exon 13	c.2090 T > C	p.Val678Ala	Substitution	Missense	Mild
Exon 13	c.2102 T > C	p.Met682Thr	Substitution	Missense	Mild
Exons 14–26			Deletion	Large deletion	Severe
Exon 14	c.2161 A > G	p.Met702Val	Substitution	Missense	Severe
Exon 14	c.2202 delT	p.Thr715del	Deletion	Small deletion	Severe
Exon 13	c.2032_37 delAAACAC	p.Lys659_His660del	Deletion	Small deletion	Severe
Exon 14	c.2216 delA	p.Glu720del	Deletion	Small deletion	Severe
Exon 14	c.2521_30 delCCTGGAGCAA	p.Pro822_Ile825del	Deletion	Small deletion	Severe
Exon 14	c.2534_37 delACAG	p.Asp826_Ser827del	Deletion	Small deletion	Severe
Exon 14	c.2729_30 insC	p.Ser891ins	Insertion	Insertion	Severe
Exon 14	c.2742 T > G	p.Leu859Stop	Substitution	Nonsense	Severe
Exon 14	c.2777_78 insC	p.Pro907ins	Insertion	Insertion	Severe
Exon 14	c.2810 delA	p.Asp918del	Deletion	Small deletion	Severe
Exon 14	c.3113 C > G	p.Ser1019Stop	Substitution	Nonsense	Severe
Exon 14	c.3224 delC	p.Ala1056del	Deletion	Small deletion	Severe
Exon 14	c.3381 G > A	p.Trp1108Stop	Substitution	Nonsense	Severe
Exon 14	c.3430 delA	p.Ser1125del	Deletion	Small deletion	Severe
Exon 14	c.3619_20 delCA	p.His1188del	Deletion	Small deletion	Severe
Exon 14	c.3625_26 insC	p.Asn1189ins	Insertion	Insertion	Severe
Exon 14	c.3652 delG	p.Glu1199del	Deletion	Small deletion	Severe
Exon 14	c.3834 delA	p.Arg1260del	Deletion	Small deletion	Severe
Exon 14	c.3865 A > T	p.Lys1270Stop	Substitution	Nonsense	Severe
Exon 14	c.3904 delCAAAC	p.Gln1282_Asn1283del	Deletion	Small deletion	Severe
Exon 14	c.3911_12 insA	p.Lys1285ins	Insertion	Insertion	Severe
Exon 14	c.3984_85 insA	p.Arg1310ins	Insertion	Insertion	Severe
Exon 14	c.4067 C > A	p.Ser1337Stop	Substitution	Nonsense	Severe
Exon 14	c.4077 G > A	p.Trp1340Stop	Substitution	Nonsense	Severe
Exon 14	c.4097 delT	p.Lys1347 del	Deletion	Small deletion	Severe
Exon 14	c.4114 A > G	p.Thr1353Ala	Substitution	Missense	Severe
Exon 14	c.4156 C > T	p.Gln1367Stop	Substitution	Nonsense	Severe
Exon 14	c.4363 C > T	p.Gln1436Stop	Substitution	Nonsense	Severe
Exon 14	c.4388_91 delCTTT	p.Ser1444_Leu1445del	Deletion	Small deletion	Severe
Exon 14	c.4477_80 delAAAG	p.Lys1474_Val1475del	Deletion	Small deletion	Severe
Exon 14	c.4483 delG	p.Glu1476del	Deletion	Small deletion	Severe
Exon 14	c.4483 G > T	p.Glu1476Stop	Substitution	Nonsense	Severe
Exon 14	c.4687 delG	p.Val1544del	Deletion	Small deletion	Severe
Exon 14	c.4774_75 insA	p.Thr1573ins	Insertion	Insertion	Severe

Table 1 continued

Location	Nucleotide	Protein	Type	Effect	Clinical phenotype
Exon 14	c.4788_89 insA	p.Glu1578ins	Insertion	Insertion	Severe
Exon 14	c.4825_26 insA	p.Thr1590ins	Insertion	Insertion	Severe
Exon 14	c.4895_96 insT	p.Ile1613ins	Insertion	Insertion	Severe
Exon 14	c.4921_22 insT	p.Ile1622ins	Insertion	Insertion	Severe/moderate
Exon 14	c.5063 C > T	p.Ser1669Leu	Substitution	Missense	Moderate
Exon 14	c.5125 delA	p.Ser1690del	Deletion	Small deletion	Severe
Exon 14	c.5176 T > C	p.Trp1707Arg	Substitution	Missense	Severe
Exon 14	c.5184 T > A	p.Tyr1709Stop	Substitution	Nonsense	Severe
Exon 14	c.5213_16 delGAAA	p.Arg1719_Asn1720del	Deletion	Small deletion	Severe
Intron 14	c.5219 + 1 G > A		Substitution	Splicing	Severe
Intron 14	c.5219 + 2 T > C		Substitution	Splicing	Severe
Intron 14	c.5220 – 2 A > T		Substitution	Splicing	Severe
Exon 15	del 3bp inser22bp	p.Val1727delins22bp	Complex	Insertion/small deletion	Severe
Exon 15	c.5253A > T	p.Lys1732Asn	Substitution	Missense	Severe
Exon 15	c.5275 G > A	p.Asp1740Asn	Substitution	Missense	Mild
Exon 15	c.5321 A > G	p.His1755Arg	Substitution	Missense	Severe
Intron 16	c.5373 + 1 G > A		Substitution	Splicing	Severe
Intron 16	c.5373 + 2 T > G		Substitution	Splicing	Moderate
Exon 16	c.5387 A > G	p.Asn1777 Ser	Substitution	Missense	Severe
Exon 16	c.5398 C > A	p.Arg1781Ser	Substitution	Missense	Severe
Exon 16	c.5398 C > T	p.Arg1781Cys	Substitution	Missense	Moderate
Exon 16	c.5405 A > C	p.Tyr1783Arg	Substitution	Missense	Severe
Exon 16	c.5413 T > A	p.Tyr1786Asn	Substitution	Missense	Severe
Exon 16	c.5419 A > G	p.Ser1788Gly	Substitution	Missense	Mild
Exon 16	c.5473_5478 delTTTGTC	p.Phe1806_Val1807del	Deletion	Small deletion	Severe
Exon 14	c.5501_5502 insA	p.Trp1815ins	Insertion	Insertion	Severe
Exon 16	c.5515C > T	p.Gln1820Stop	Substitution	Nonsense	Severe
Exon 16	c.5522 A > T	p.His1822Leu	Substitution	Missense	Mild
Exon 16	c.5561 T > G	p.Trp1835Gly	Substitution	Missense	Severe
Exon 17	c.5662 A > T	p.Arg1869Stop	Substitution	Nonsense	Severe
Exon 17	c.5674 G > A	p.Val1873Ile	Substitution	Missense	Moderate
Exon 17	c.5688_89 delCT	p.Leu1878del	Deletion	Small deletion	Moderate
Exon 17	c.5715 G > A	p.Gly1850Asp	Substitution	Missense	Severe
Exon 18	c.5824 G > A	p.Gly1923Asp	substitution	Missense	Severe
Exon 18	c.5837 A > T	p.Asp1927Val	substitution	Missense	Mild
Exon 18	c.5918 A > G	p.His 1954 Arg	Substitution	Missense	Severe
Exon 18	c.5998 G > A	p.Gly1981Ser	Substitution	Missense	Severe
Exon 19	c.6043 T > C	p.Trp1996Arg	Substitution	Missense	Severe
Exon 19	c.6104 T > G	p.Val2016Gly	Substitution	Missense	Severe
Intron 19	c.6115 + 4 A > G		Substitution	Splicing	Severe
Intron 19	c.6115 delGTG + 1_+3		Substitution	Splicing	Severe
Exon 19	c.6151 A > T	p.Ile2032Phe	Substitution	Missense	Mild
Intron 21	c.6187 – 1 G > A		Substitution	Splicing	Severe
Exon 21	c.6267 G > T	p.Trp2070Cys	Substitution	Missense	Mild
Exon 22	c.6310 A > T	p.Lys2085Stop	Substitution	Nonsense	Severe
Exon 22	c.6312_13 insG	p.Thr2086ins	Insertion	Insertion	Severe
Exon 22	c.6320 G > A	p.Gly2088Asp	Substitution	Missense	Severe

Table 1 continued

Location	Nucleotide	Protein	Type	Effect	Clinical phenotype
Exon 21	c.6335 G > T	p.Gly2057Stop	Substitution	Nonsense	Severe
Exon 22	c.6374 G > C	p.Ser2106Thr	Substitution	Missense	Severe
Intron 22	c.6430 –21 + c.6430_6439 del		Deletion	Small deletion	Severe
Exon 23	c.6506 G > T	p.Arg2150Leu	Substitution	Missense	Mild
Exon 23	c.6546T > C	p.Phe2127Ser	Substitution	Missense	Mild
Exon 23	c.6560 G > A	p.Gly2168Asp	Substitution	Missense	Severe
Exon 23	c.6563 G > A	p.Cys2169Tyr	Substitution	Missense	Moderate
Exon 24	c.6593 G > A	p.Gly2179Glu	Substitution	Missense	Severe
Exon 24	c.6593 G > T	p.Gly2179Val	Substitution	Missense	Severe
Exon 24	c.6623_25 delAGA Gln2189 + c.6628 delA Thr2191	p.Gln2189del + p.Thr2191del	Complex	Small deletions	Severe
Exon 24	c.6637 T > C	p.Ser2194Pro	Substitution	Missense	Severe
Exon 24	c.6638 C > G	p.Ser2194Cys	Substitution	Missense	Moderate
Exon 24	c.6650 delA	p.Asn2198del	Deletion	Small deletion	Severe
Exon 24	c.6652 A > G	p.Met2199Val	Substitution	Missense	Severe
Exon 25	c.6738 G > A	p.Glu2228Lys	Substitution	Missense	Moderate
Exon 25	c.6739 G > T	p.Glu2228Stop	Substitution	Nonsense	Severe/moderate
Exon 25	c.6742 T > C	p.Trp2229Arg	Substitution	Missense	Mild
Exon 25	c.6746 T > C	p.Leu2230Pro	Substitution	Missense	Severe
Exon 25	c.6776 T > C	p.Val2240Asp	Substitution	Missense	Severe
Exon 25	c.6785 T > C	p.Val2243Ala	Substitution	Missense	Moderate
Exon 25	c.6894_95 insAAC	p.Val1280ins	Insertion	Insertion	Severe
Exon 26	c.6907 C > T	p.Gln2284Stop	Substitution	Nonsense	Severe
Exon 26	c.6956 C > G	p.Pro2300Arg	Substitution	Missense	Severe
Exon 26	c.6966_67 insGACT	p.Thr2303ins	Insertion	Insertion	Severe
Exon 26	c.6982 C > G	p.His2309Asp	Substitution	Missense	Severe
Exon 26	c.6995 G > C	p.Trp2313Ser	Substitution	Missense	Moderate
Exon 26	c.7015 A > T	p.Arg2320Trp	Substitution	Missense	Severe
Exon 26	c.*1 T > C		Substitution	Missense	Mild

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