

ARTICLE

Male and female differential reproductive rate could explain parental transmission asymmetry of mutation origin in Hirschsprung disease

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Hirschsprung disease (HSCR, aganglionic megacolon) is a complex and heterogeneous disease with an incidence of 1 in 5000 live births. Despite the multifactorial determination of HSCR in the vast majority of cases, there is a monogenic subgroup for which private rare *RET* coding sequence mutations with high penetrance are found (45% of HSCR familial cases). An asymmetrical parental origin is observed for *RET* coding sequence mutations with a higher maternal inheritance. A parent-of-origin effect is usually assumed. Here we show that a differential reproductive rate for males and females also leads to an asymmetrical parental origin, which was never considered as a possible explanation till now. In the case of HSCR, we show a positive association between penetrance of the mutation and parental transmission asymmetry: no parental transmission asymmetry is observed in sporadic *RET* CDS mutation carrier cases for which penetrance of the mutation is low, whereas a parental transmission asymmetry is observed in affected sib-pairs for which penetrance of the mutation is higher. This allows us to conclude that the explanation for this parental asymmetry is that more severe mutations have resulted in a differential reproductive rate between male and female carriers.

European Journal of Human Genetics (2012) 20, 917–920; doi:10.1038/ejhg.2012.35; published online 7 March 2012

Keywords: Hirschsprung disease; parent-of-origin effect; parental transmission asymmetry; reproductive rate

INTRODUCTION

Hirschsprung disease (HSCR, aganglionic megacolon) is the most frequent genetic cause of congenital intestinal obstruction. The *RET* gene, coding for a tyrosine kinase receptor, is implicated in all HSCR cases, but two subgroups of patients have to be distinguished. A small subgroup of patients present a rare coding sequence mutation (CDS) of the *RET* gene with a high and sex-dependent penetrance.^{1,2} The remaining group of patients have a risk allele for a noncoding polymorphism in an enhancer element of IVS1, rs2435357, with a low and sex-dependent penetrance^{3–6} (found in 90% of the patient alleles *versus* 19.4% in the 1000 genomes project). The subgroup with *RET* CDS mutations represents ~45% of familial cases and 7–20% of sporadic cases.⁴

Bolk *et al*¹ observed that the shared allele at *RET* among affected sib-pairs is more frequently inherited from the mother than the father. As families with and without *RET* CDS mutation were pooled, the question of whether parental transmission asymmetry is observed in both types of families remains unanswered. As 35% of families studied in Bolk *et al*¹ paper carry a *RET* CDS mutation, we

hypothesized that the parental transmission asymmetry observed in this sample could be the consequence of a high rate of *RET* CDS mutations only. As rs2435357 was not yet identified as a risk variant at the time of the study in the sample reported in the paper of Bolk *et al*,¹ we had to check this hypothesis in another sample. We thus analyzed the data set from the International Hirschsprung Consortium that had been characterized for both *RET* CDS mutations and rs2435357. This sample includes some of the families previously studied in Bolk *et al*.¹ Here, 490 patients were checked for parental origin of rs2435357 risk allele and there was no statistical evidence for preferential paternal or maternal transmission (242 paternal *versus* 249 maternal transmissions). However, for the 39 patients with an inherited *RET* CDS mutation and for whom parental origin was available, a parental transmission asymmetry was observed, with 14 mutations paternally inherited and 25 maternally inherited. We therefore focused in the present paper on the subgroup of patients with *RET* CDS mutations to question the observed parental transmission asymmetry found for *RET* CDS mutations.

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Received 8 July 2011; revised 17 January 2012; accepted 31 January 2012; published online 7 March 2012

Parent-of-origin effect, defined by the manifestation of the disease in the offspring depending on the sex of the transmitting parent, is the usual explanation for an observed parental transmission asymmetry. However, a differential reproductive rate among males and females carrying a *RET* CDS mutation is also an explanation to consider for Hirschsprung disease. Sex ratio in HSCR is indeed unbalanced with more affected males (in our sample, 14 females for 25 males). As *RET* gene is autosomal, the same number of females and males carry the mutation at conception. Therefore, penetrance differs for male and female mutations carriers. Males carrying a *RET* CDS mutation are more likely to be affected. Consequently, because of the poor prognosis of the disease until recently, males are less likely to reproduce than females carrying a *RET* CDS mutation. Thus, although the same proportion of males and females carry a *RET* CDS mutation at conception, more females than males would be observed among reproducing adults carrying a *RET* CDS mutation. At the next generation, we would therefore observe more *RET* CDS mutation inherited from the mother than from the father. This would result in an asymmetrical parental transmission origin.

A differential reproductive rate for males and females due to high and sex-dependent penetrance of a mutation has never been considered in the literature as a possible explanation for a parental transmission asymmetry. Many other ascertainments and mechanisms explaining an asymmetrical parental origin have largely been discussed.^{7–10} However, this literature deals with parental transmission asymmetry observed for common variant with low penetrance, and a differential reproductive rate for males and females cannot be an explanation in these situations.

Parental transmission asymmetry for *RET* CDS mutations in HSCR cannot be discussed without considering the mutation spectrum observed as private mutation is the rule. These mutations have indeed a large penetrance spectrum. In the case of a mutation with low penetrance, most *RET* CDS mutation carriers would not be affected and therefore would reproduce. On the contrary, for mutation with near-complete penetrance, as the disease sex ratio is unbalanced, more healthy females than males would be observed, more females than males would reproduce, and, therefore, parental transmission

asymmetry would be observed at the next generation. A link between parental transmission asymmetry and penetrance of the mutation would thus be a strong argument in favor of a differential reproductive rate explaining parental transmission asymmetry. Indeed, such a correlation has never been described and will not be observed in the case of a parent-of-origin effect.

The goal of this paper is to test whether a differential reproductive rate explains the observed parental transmission asymmetry for *RET* CDS mutations, and more specifically to test if parental transmission asymmetry is correlated with penetrance of the mutation. In this aim, we take advantage of a unique worldwide sample of patients from the International Consortium on Hirschsprung Disease, gathering both sporadic cases and affected sib-pairs to study the correlation between parental transmission asymmetry and penetrance of the mutation. We also explore a possible complementary role of rs2435357 on parental transmission asymmetry.

MATERIALS AND METHODS

The data used in this paper have been obtained by the International Hirschsprung Disease Consortium. In this data set, CDS mutations have been searched for in 655 individuals and identified in 94 families, and among them were 21 affected sib-pairs and 73 sporadic cases (see Figure 1). Families from the US and Spanish data set have already been published.^{2,11,12}

Because parental DNA was not available for all *RET* CDS mutation carriers patients, parental origin of *RET* CDS mutations was only searched for in 18 affected sib-pairs and 36 sporadic cases. A total of 15 *de novo* mutations were detected for sporadic cases. The parental origin of mutation was thus available for 21 sporadic cases. All information about these samples is summarized in Supplementary Table S1 and in Figure 1. Only two affected individuals had an affected parent (families 73 and 134, both from France).

Penetrances of the mutations are expected to be lower in sporadic cases than in sib-pairs. However, the classical approach of using the recurrence risk in the sibships of probands cannot be used as sibship sizes are not available in our sample and as most mutations are private. Consequently, to assess the fact that penetrances of the mutations are lower in sporadic cases than in affected sib-pairs, we used the information on the mutation position. Mutations within the extracellular domain are considered to disrupt *RET* maturation/folding that subsequently fails to pass the endoplasmic reticulum quality control,¹³ leading to a more drastic loss of function than mutations lying in the transmembrane

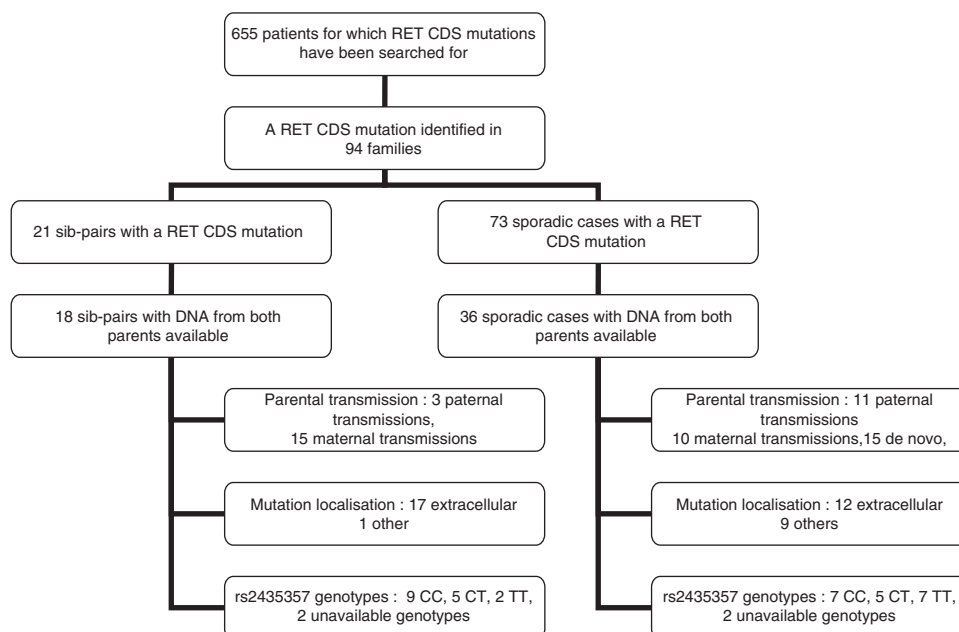


Figure 1 Diagram describing sample constitution of HSCR patients included in this study.

and intracellular domain. Consequently, we considered that mutations within the extracellular domain have a higher penetrance than other mutations.

Thus, we tested, in a first step, whether the proportion of mutations within the extracellular domain was greater in affected sib-pairs than in sporadic cases and, in a second step, whether the parental transmission asymmetry was greater in affected sib-pairs and sporadic cases. Both tests were performed using the χ^2 test.

As mutations penetrance was not complete (only two affected parents), we suspect that other factors on *RET* gene could modulate penetrance. We tested whether the risk allele frequency of rs2435357, known to have an increased frequency in *RET* mutation carrier HSCR patients, was more frequent in sporadic cases than in affected sib-pairs. Indeed, if rs2435357 risk allele frequency was decreased in affected sib-pairs compared with sporadic cases, this would be a further argument for penetrance of the mutation to be greater in affected sib-pairs than in sporadic cases. We also tested whether the risk allele *in trans* was more frequent in affected patients than in their unaffected parent in order to explain at least partly why most parents were unaffected.

All calculations have been performed using R software.¹⁴

RESULTS

There were 17 extracellular mutations *versus* only one other mutation for affected sib-pairs, whereas extracellular and other mutations had similar frequencies (12 extracellular *versus* 9 other mutations) for sporadic cases. The proportion of extracellular mutation was therefore significantly lower in sporadic and affected sib-pairs ($P=0.02$), with sib-pairs having mutations with a higher penetrance.

Among sporadic cases, the *RET* CDS mutant allele was equally inherited from the mother and father (10 *versus* 11), whereas in affected sib-pairs there was a strong parental transmission asymmetry (15 from maternal origin *versus* 3 from paternal origin), giving a different parental transmission asymmetry among these samples ($P=0.05$). Therefore, this positive association between penetrance of the mutation and parental transmission asymmetry allows us to conclude that the explanation for this asymmetry is that more severe mutations have resulted in a differential reproductive rate between male and female carriers.

We then tested the additional role of rs2435357 (cf, Supplementary Table S1). The difference of frequency for rs2435357 risk allele between affected sib-pairs and sporadic cases was in the expected direction but only suggestive (9 out of 32 for sib-pairs *versus* 19 out of 38 for sporadic cases, $P=0.1$). We tested whether this polymorphism was more frequent in affected CDS mutation carriers than in their unaffected CDS mutation carrier parents. Interestingly, affected children carried the hypomorphic T allele in *trans* position more frequently than their unaffected CDS mutation carrier parents (12 out of 27 for children *versus* 3 out of 23 for carrier parents, $P=0.04$, see Supplementary Table S1).

DISCUSSION

We face two situations: sporadic cases for which no parental transmission asymmetry is observed and affected sib-pairs for which a strong parental transmission asymmetry is observed. This observation is positively correlated with the proportion of extracellular mutations in each sample. This result favors differential reproductive rates for male and female mutation carriers explaining the observed parental asymmetrical origin. We also underline here the complementary role of rs2435357 on *RET* CDS mutations with the risk allele in *trans* increasing penetrance. Therefore, the genotype of both parents affect *RET* CDS mutation penetrance in children, explaining why former segregation study results could not clearly distinguish between a dominant or recessive mode of inheritance.¹⁵

This study proposes to classify mutations using their localization. Indeed, a classification of mutations was not straightforward as

mutations are private and only very few have been studied *in vitro*.^{13,16} Meanwhile, the classification that we propose here should not be used by clinicians as more specific classifications exist for some mutations,^{13,16} and this classification has no value for a particular mutation as some other features such as location on a binding site might increase strongly penetrance. For instance, one of the two paternally transmitted mutations in sibs in the extracellular domain was a missense change (p.Cys609Thr, family 88 from United States), known for its low penetrance. The other paternally transmitted mutation in sibs (p.Arg231His, family 49 from France) was a missense changing a basic histidine residue into another basic arginine residue in the extracellular domain, presumably of mild effect on the *RET* protein. Along the same lines, the only intracellular mutation found in a sib-pair (p.Met1064Thr, family 45 from France) affects codon 1064 close to the major phosphorylation site of the *RET* receptor (Tyr1062). Hence, that mutation, despite being located in the intracellular domain, may lead to a loss of function similar to the ones lying in the extracellular *RET* domain.

Other mechanisms commonly assumed when observing parental transmission asymmetry do not support a correlation between penetrance and parental transmission asymmetry or would involve mechanisms never described until yet to explain such correlation. If imprinting was involved, then we had to assume a differential imprinting among *RET* CDS mutations to explain such correlation. Such a situation has never been described in the literature. Anyway, imprinting is unlikely as the *RET* gene does not map to a known imprinted locus, although tissue-specific imprinting remains possible as demonstrated for the *UBE3A*¹⁷ or *GNAS* genes.¹⁸ A further attractive hypothesis was sex-specific genomic imprinting that would explain both the asymmetrical parental origin and the observed sex ratio. A sex-specific genomic imprinting has been found to be far more frequent than commonly assumed in mouse brain.¹⁹ However, this hypothesis was unlikely for HSCR as *RET* expression is biallelic in the gut.²⁰ The question of a sperm-specific fitness explaining the parental transmission asymmetry, that is, a selective advantage for spermatozooids not carrying a *RET* CDS mutation, could also be raised, as the *RET* gene is known to play a crucial role in early spermatogenesis.²¹ However, a selective advantage,²² and not disadvantage, has been described for spermatogonia carrying a *RET* mutation.

This study is a further argument against parental transmission asymmetry and the disease sex ratio in favor of females to be positively associated. Indeed, the sex ratio observed decreases with parental transmission asymmetry from 15:6 in sporadic case to 10:8 in affected sib-pairs. Therefore, the sex ratio decreases while parental transmission asymmetry increases. Furthermore, sex ratio decreases when the frequency of the *RET* CDS mutation increases; in a large sample of 882 probands with 13% of *RET* gene CDS mutations,⁵ the sex ratio was close to 5 whereas it dropped to 2 in our CDS mutation carriers sample. This prompts us to search for factor(s) other than *RET* gene responsible for the skewed sex ratio observed in HSCR.

It is likely that some genes involved in diseases have been suspected of a parent of origin, although sex-biased penetrance could also explain the observed data. Considering cleft lip with or without cleft palate, the sex ratio is biased with more affected males than females and an excess of maternal transmission in sporadic cases has been reported for several disease-causing genes.^{23–26} As cleft lip may harbor a social disadvantage and more males than females are affected, reduced fertility of affected individuals could lead to the observation of an excess of maternal transmission. Another example is hereditary cystatin C amyloid angiopathy (HCCAA), which is an autosomal dominant disease with high penetrance, responsible for brain

hemorrhages in young normotensive adults. Intriguingly, the genealogies reveal that L68Q carriers experience a drastic reduction in lifespan from 65 years in the past to 30 years at present.²⁷ During the same time period, a parental transmission asymmetry has emerged, whereby maternal inheritance of the mutation is associated with a 9-year reduction in lifespan relative to paternal inheritance. The reduction in lifespan combined with the emergence of an asymmetrical parental origin could be explained by the combination of a lower reproductive rate for patients and a higher disease penetrance in males compared with females. It could also explain why hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) show an excess of mortality when the mutation is paternally inherited.²⁸ Indeed, as this disease is more severe in females than in males, fewer female mutation carriers are likely to reach adulthood than males.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study is a contribution from The International Hirschsprung Disease Consortium. We thank the numerous patients, families and referring physicians across the world who have participated in studies of Hirschsprung disease in our laboratories. This work was funded by the French National Research Agency (ANR, grants MRARE-HirGenet to SL, and ERare-HCR Consortium to SL, IC, and SB), the Fondation pour la Recherche Médicale (FRM) to SL and JA; the National Institutes of Health (R37 HD28088 to AC); the Italian Telethon (GGP04257 to IC); the Fondo de Investigación Sanitaria, Spain PI1001290 and Consejería de Economía, Innovación y Ciencia de la Junta de Andalucía (CTS7447) to SB; the NWO (901-04-225) Bernoulle Foundation and Ubbo Emmius Foundation to RMWH; and the Hong Kong Research Grant Council (HKU 765407 to MG-B and PKHT).

WEB RESOURCES

The URL for data presented herein is as follows: 1000 Genomes project, <http://www.1000genomes.org/>.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)